US009162992B2

(12) **United States Patent**
Mannion et al.

(10) **Patent No.: US 9,162,992 B2**
(45) **Date of Patent: Oct. 20, 2015**

(54) **COMPOUNDS AND COMPOSITIONS FOR TREATMENT OF BREATHING CONTROL DISORDERS OR DISEASES**

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(73) Assignee: **Galleon Pharmaceuticals, Inc.**, Horsham, PA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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(22) Filed: **Aug. 29, 2014**

(65) **Prior Publication Data**

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Related U.S. Application Data

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(60) Provisional application No. 61/494,268, filed on Jun. 7, 2011, provisional application No. 61/417,777, filed on Nov. 29, 2010.

(51) **Int. Cl.**
C07D 251/54 (2006.01)
A61K 31/505 (2006.01)
(Continued)

(52) **U.S. Cl.**
CPC ***C07D 251/54*** (2013.01); ***A61K 31/505*** (2013.01); ***A61K 31/519*** (2013.01);
(Continued)

(58) **Field of Classification Search**
CPC C07D 251/54
See application file for complete search history.

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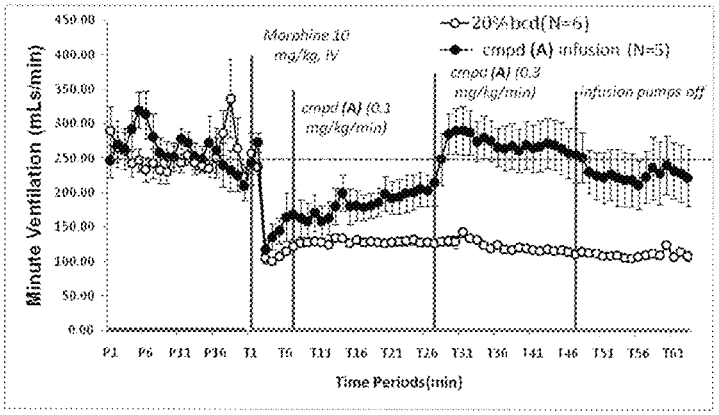
Primary Examiner — Timothy Thomas
(74) *Attorney, Agent, or Firm* — Saul Ewing LLP; Kathryn Doyle; Domingos J. Silva

(57) **ABSTRACT**

The present invention includes compounds, and compositions comprising the same, that are useful in the treatment of breathing control diseases or disorders in a subject in need thereof. The present invention also includes a method of treating a respiratory disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a compound of the invention. The present invention further includes a method of preventing destabilization or stabilizing breathing rhythm in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a compound of the invention.

33 Claims, 46 Drawing Sheets



(51) **Int. Cl.**

A61K 31/519	(2006.01)
A61K 31/522	(2006.01)
A61K 31/53	(2006.01)
A61K 31/5355	(2006.01)
A61K 31/57	(2006.01)
A61K 45/06	(2006.01)
C07D 403/12	(2006.01)
C07D 401/14	(2006.01)
C07D 413/04	(2006.01)
C07D 251/44	(2006.01)
C07D 487/04	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K31/522*** (2013.01); ***A61K 31/53*** (2013.01); ***A61K 31/5355*** (2013.01); ***A61K 31/57*** (2013.01); ***A61K 45/06*** (2013.01); ***C07D 251/44*** (2013.01); ***C07D 401/14*** (2013.01); ***C07D 403/12*** (2013.01); ***C07D 413/04*** (2013.01); ***C07D 487/04*** (2013.01)

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Fig. 1

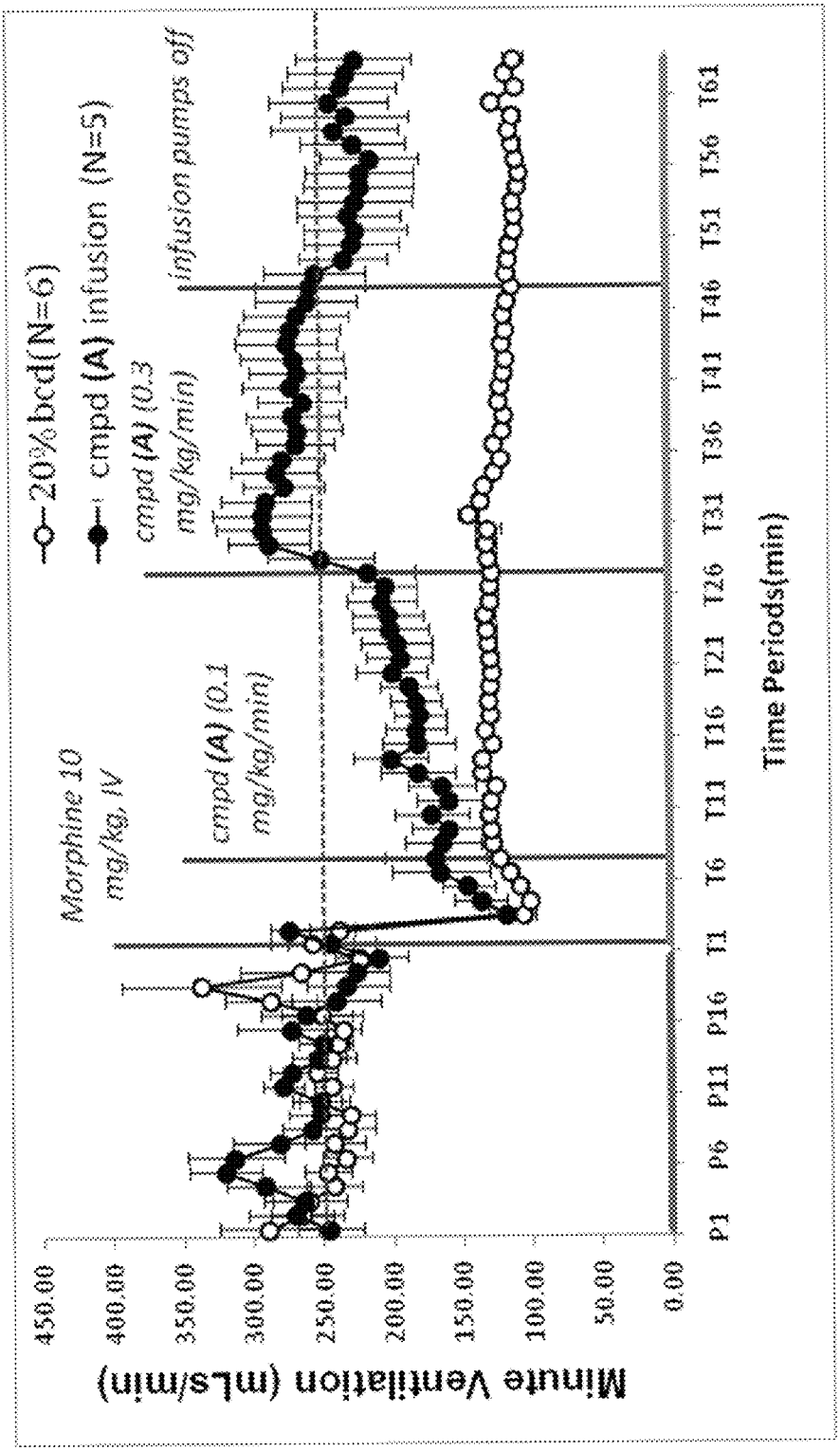

20% bcd(N=6)
cmpd (A) infusion (N=5)
Morphine 10 mg/kg, IV
cmpd (A) (0.1 mg/kg/min)
cmpd (A) (0.3 mg/kg/min)
infusion pumps off
Minute Ventilation (mLs/min)
450.00
400.00
350.00
300.00
250.00
200.00
150.00
100.00
50.00
0.00
P1 P6 P11 P16 T1 T6 T11 T16 T21 T26 T31 T36 T41 T46 T51 T56 T61
Time Periods(min)

Fig. 2A

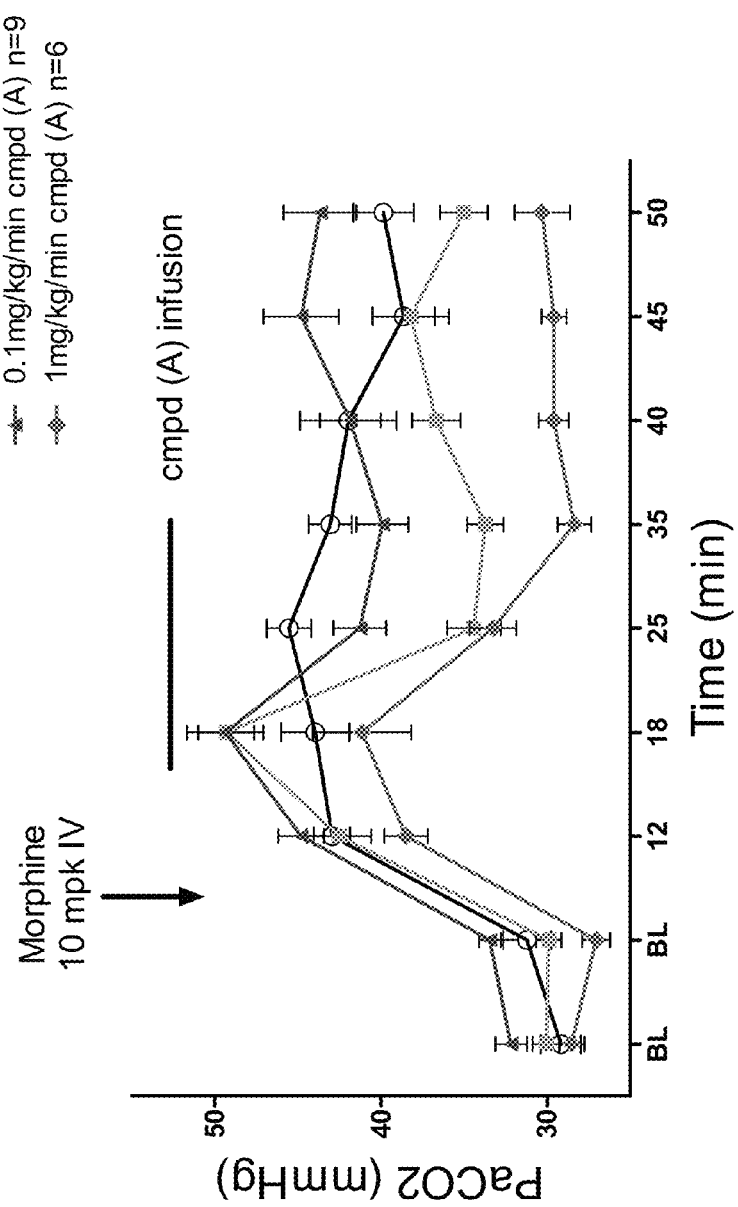

20% BCD n=10
0.3mg/kg/min cmpd (A) n=11
0.1mg/kg/min cmpd (A) n=9
1mg/kg/min cmpd (A) n=6
Morphine
10 mpk IV
cmpd (A) infusion
PaCO2 (mmHg)
50
40
30
BL
BL
12
18
25
35
40
45
50
Time (min)

Fig. 2B

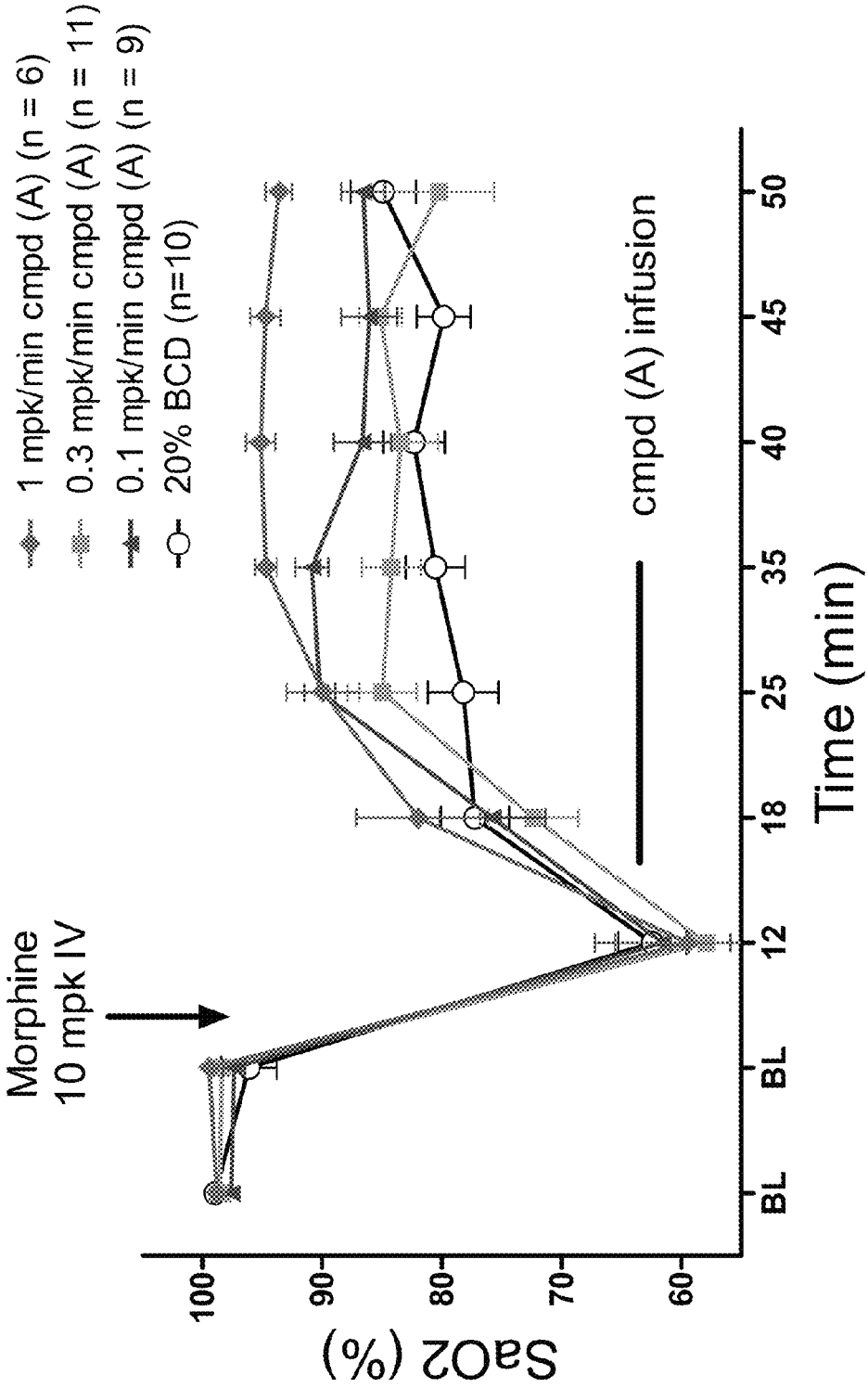

1 mpk/min cmpd (A) (n = 6)
0.3 mpk/min cmpd (A) (n = 11)
0.1 mpk/min cmpd (A) (n = 9)
20% BCD (n=10)
Morphine
10 mpk IV
cmpd (A) infusion
SaO2 (%)
100
90
80
70
60
BL
BL
12
18
25
35
40
45
50
Time (min)

Fig. 3

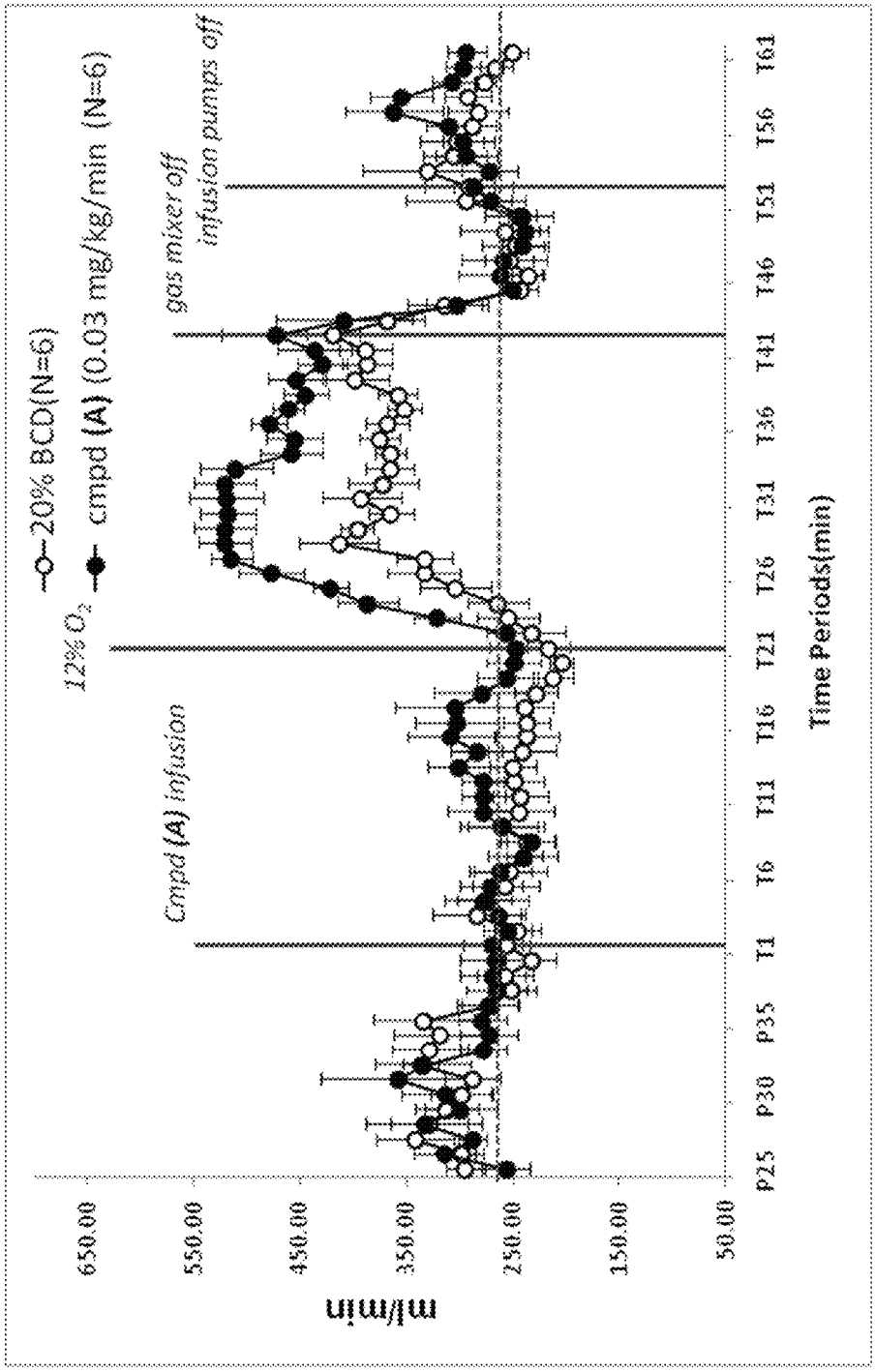

20% BCD(N=6)
cmpd (A) (0.03 mg/kg/min (N=6)
12% O_2
Cmpd (A) infusion
gas mixer off
infusion pumps off
ml/min
650.00
550.00
450.00
350.00
250.00
150.00
50.00
P25 P30 P35 T1 T6 T11 T16 T21 T26 T31 T36 T41 T46 T51 T56 T61
Time Periods(min)

Fig. 4

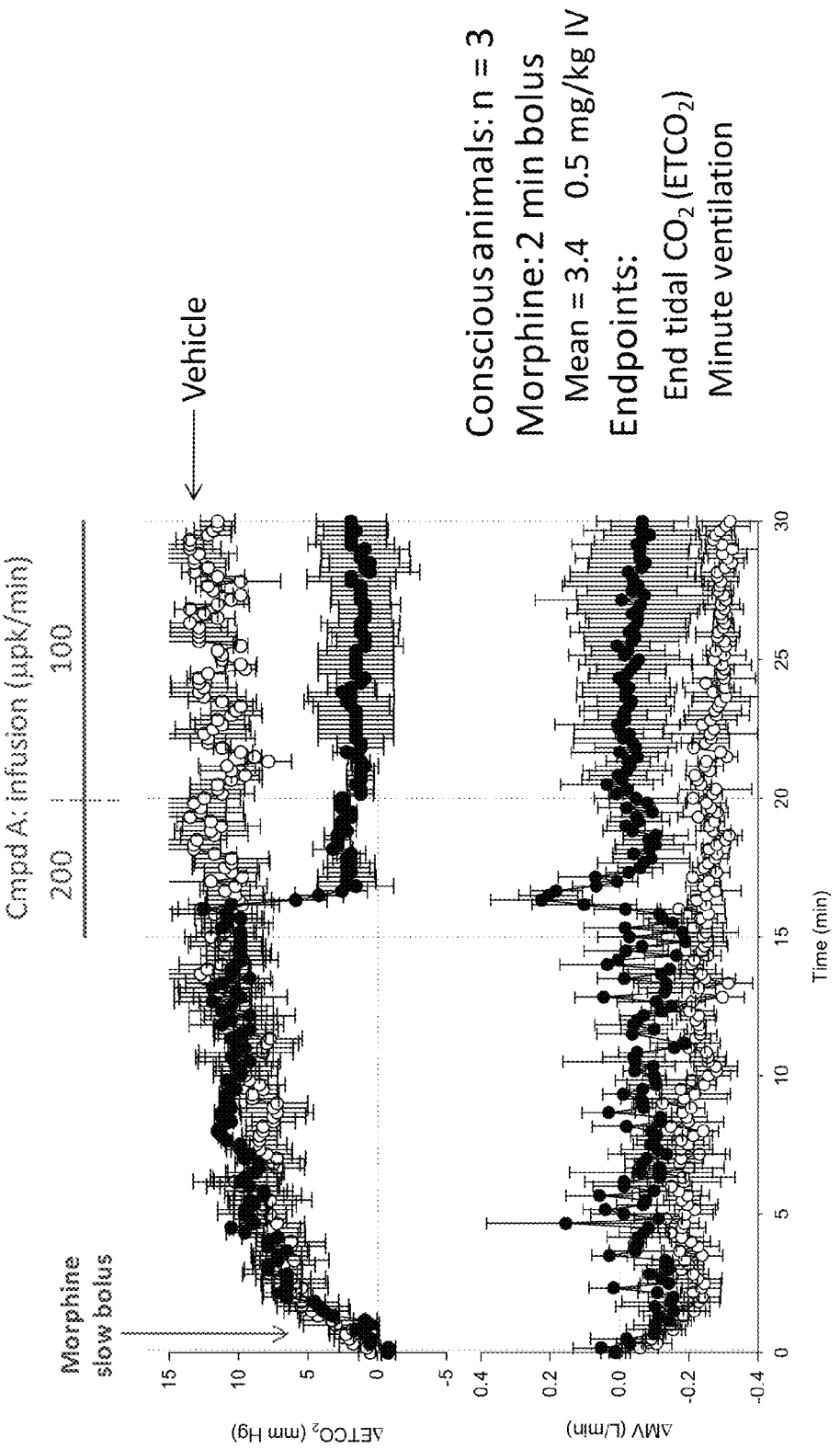

Conscious animals: n = 3
Morphine: 2 min bolus
Mean = 3.4 0.5 mg/kg IV
Endpoints:
End tidal CO_2 ($ETCO_2$)
Minute ventilation
Vehicle
Cmpd A: infusion (µpk/min)
200
100
Morphine slow bolus
$\Delta ETCO_2$ (mm Hg)
15
10
5
0
-5
ΔMV (L/min)
0.4
0.2
0.0
-0.2
-0.4
0
5
10
15
20
25
30
Time (min)

Fig. 5

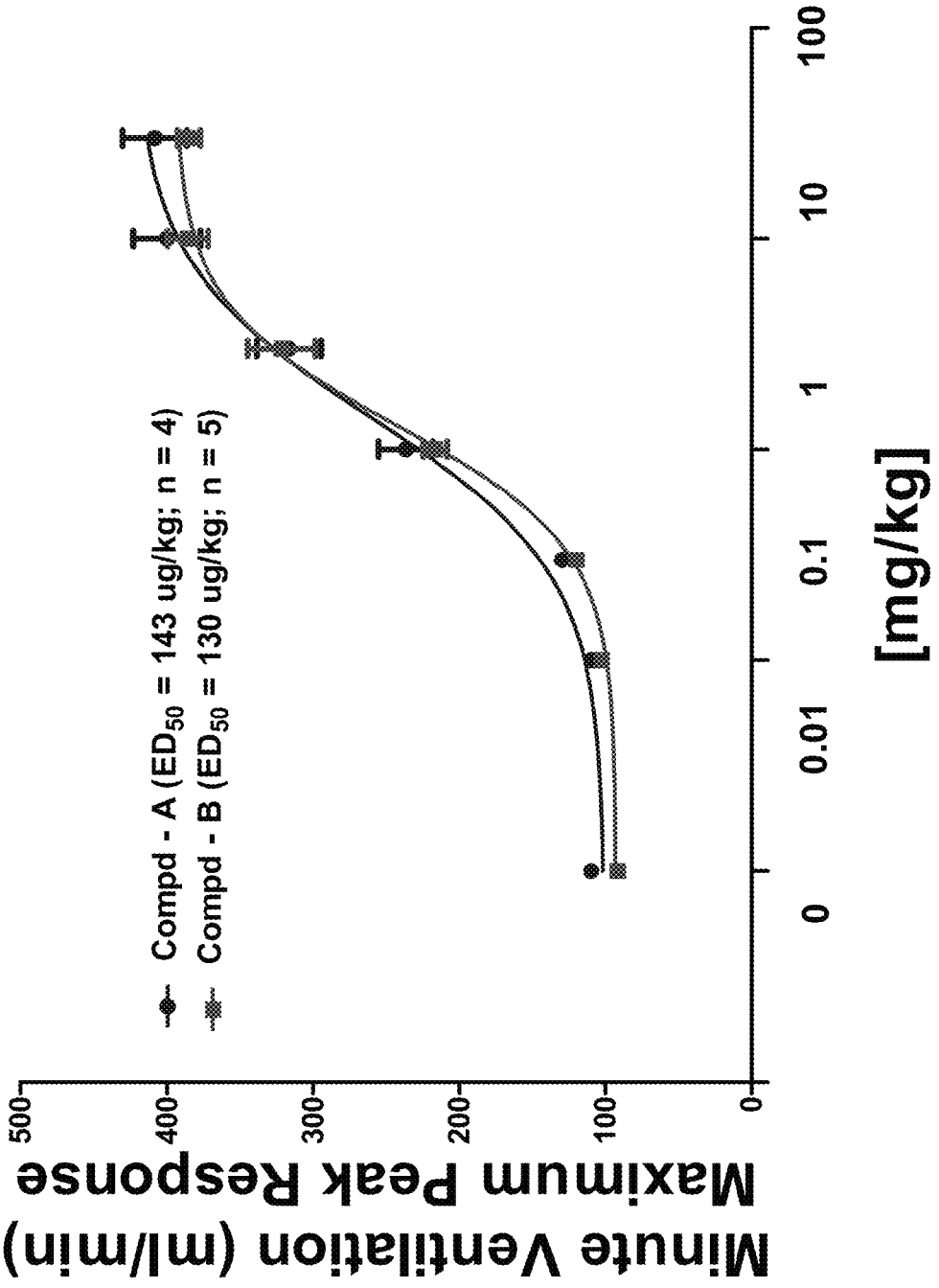

Minute Ventilation (ml/min)
Maximum Peak Response
500
400
300
200
100
0
Compd - A (ED50 = 143 ug/kg; n = 4)
Compd - B (ED50 = 130 ug/kg; n = 5)
0
0.01
0.1
1
10
100
[mg/kg]

Fig. 6

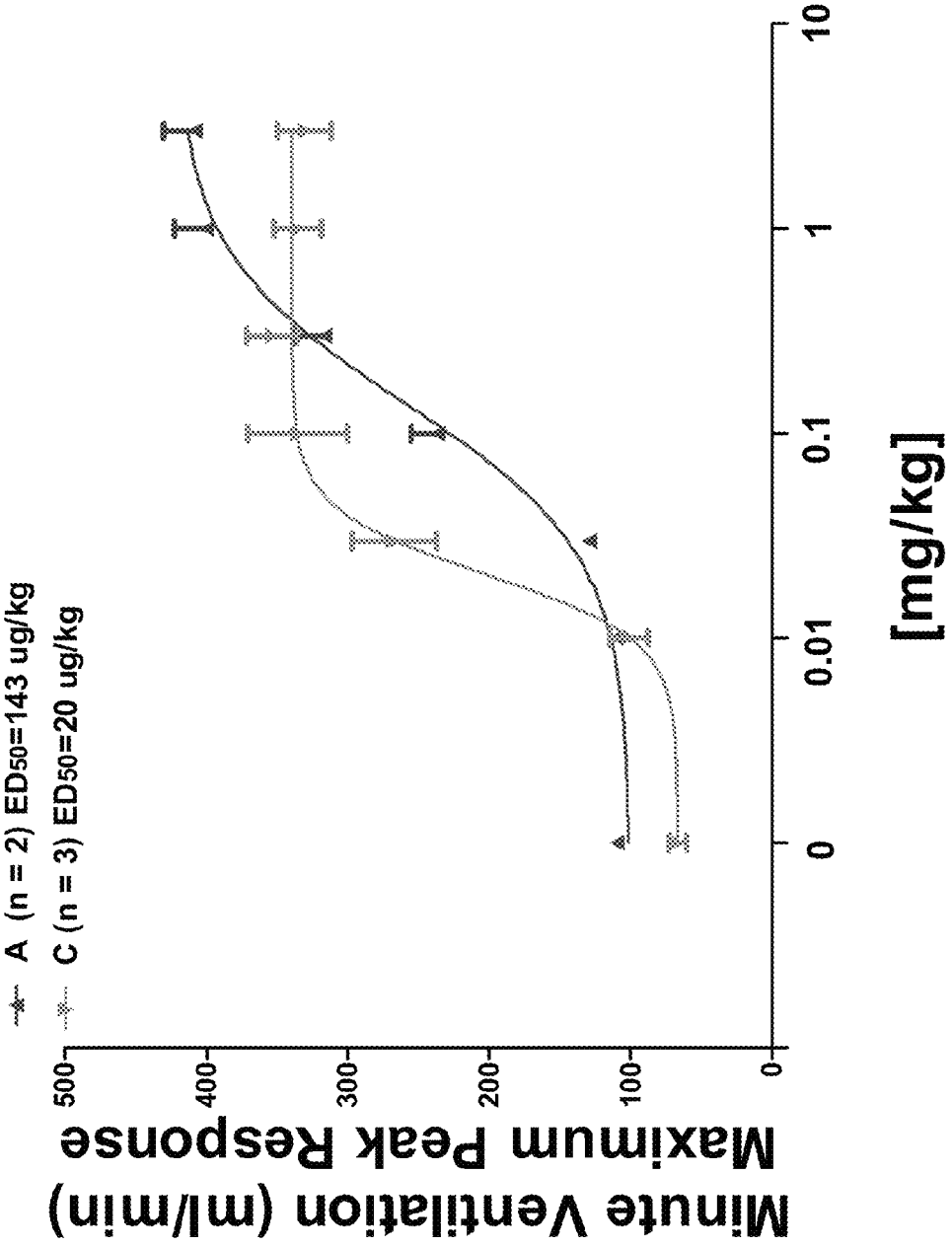

A (n = 2) ED50=143 ug/kg
C (n = 3) ED50=20 ug/kg
Minute Ventilation (ml/min)
Maximum Peak Response
500
400
300
200
100
0
0
0.01
0.1
1
10
[mg/kg]

Fig. 7A

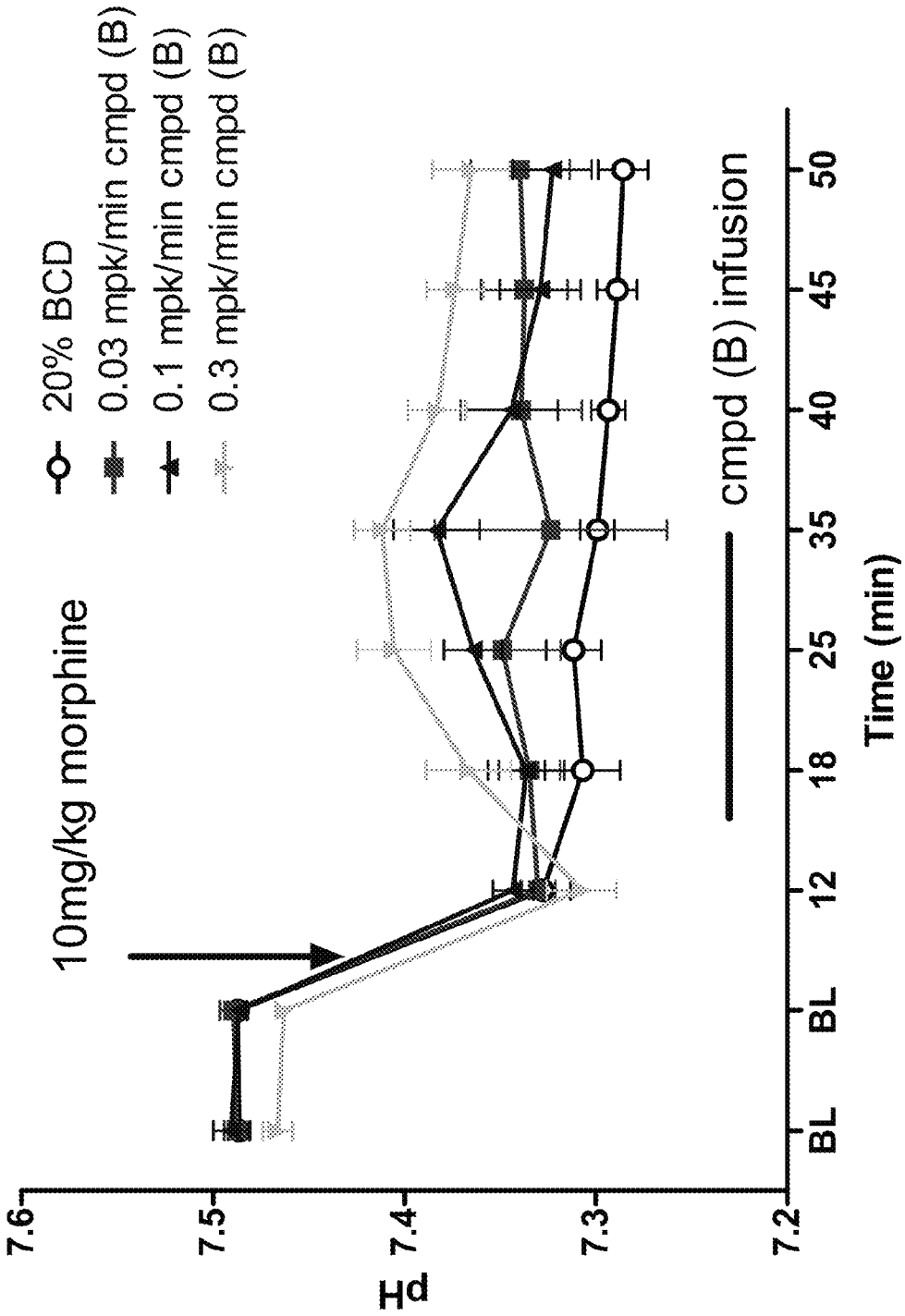

10mg/kg morphine
20% BCD
0.03 mpk/min cmpd (B)
0.1 mpk/min cmpd (B)
0.3 mpk/min cmpd (B)
cmpd (B) infusion
pH
7.6
7.5
7.4
7.3
7.2
BL
BL
12
18
25
35
40
45
50
Time (min)

Fig. 7B

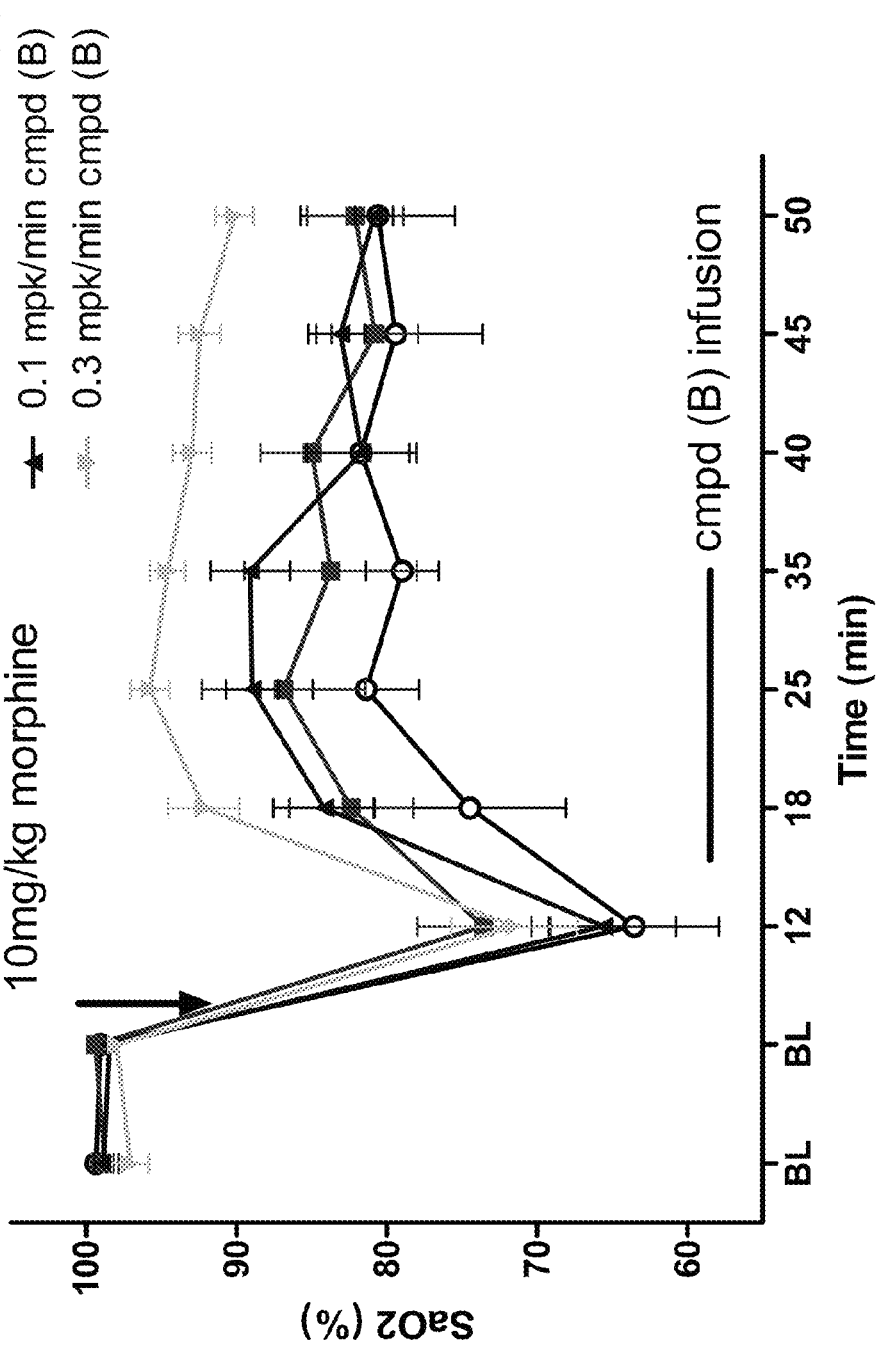

20% BCD
0.03 mpk/min cmpd (B)
0.1 mpk/min cmpd (B)
0.3 mpk/min cmpd (B)
10mg/kg morphine
cmpd (B) infusion
SaO2 (%)
100
90
80
70
60
BL
BL
12
18
25
35
40
45
50
Time (min)

Fig. 7C

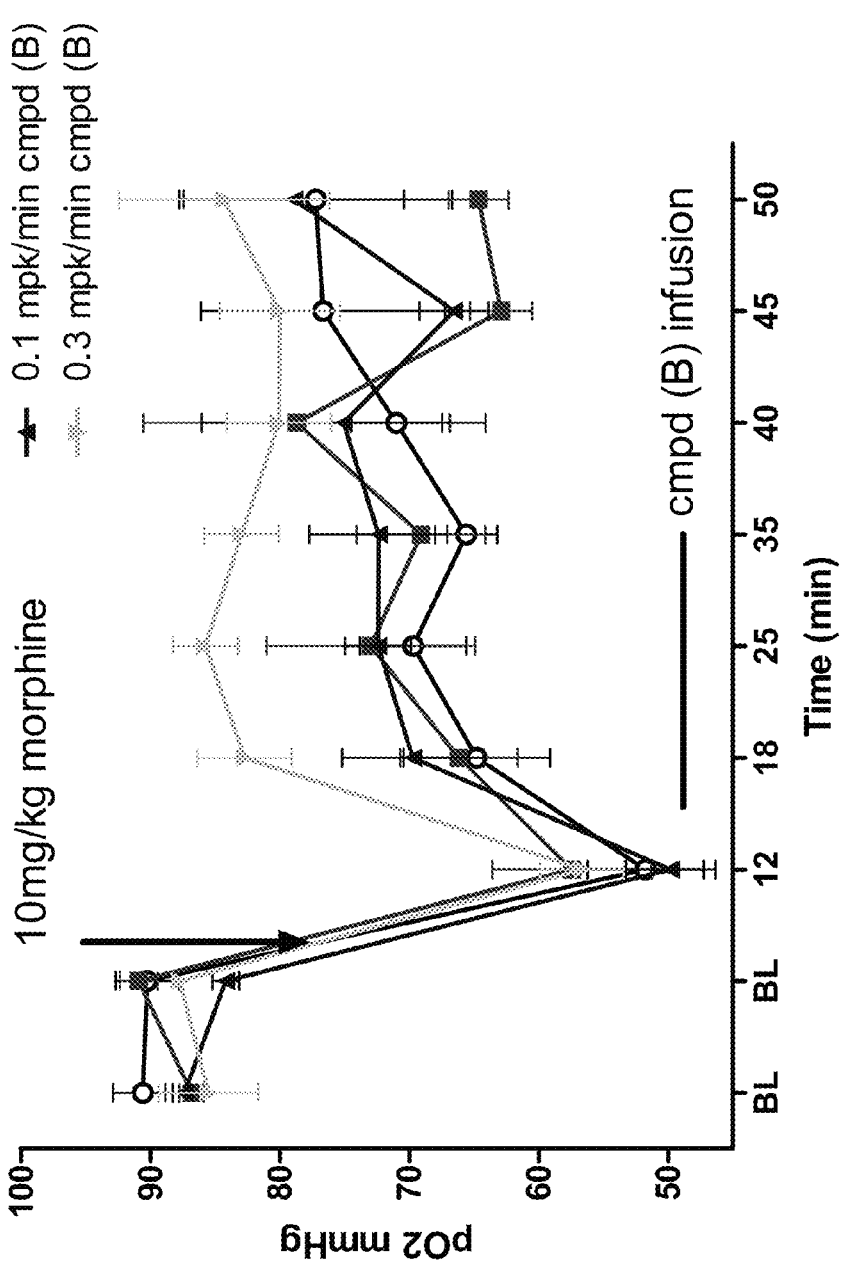

20% BCD
0.03 mpk/min cmpd (B)
0.1 mpk/min cmpd (B)
0.3 mpk/min cmpd (B)
10mg/kg morphine
cmpd (B) infusion
pO2 mmHg
Time (min)
100
90
80
70
60
50
BL
BL
12
18
25
35
40
45
50

Fig. 7D

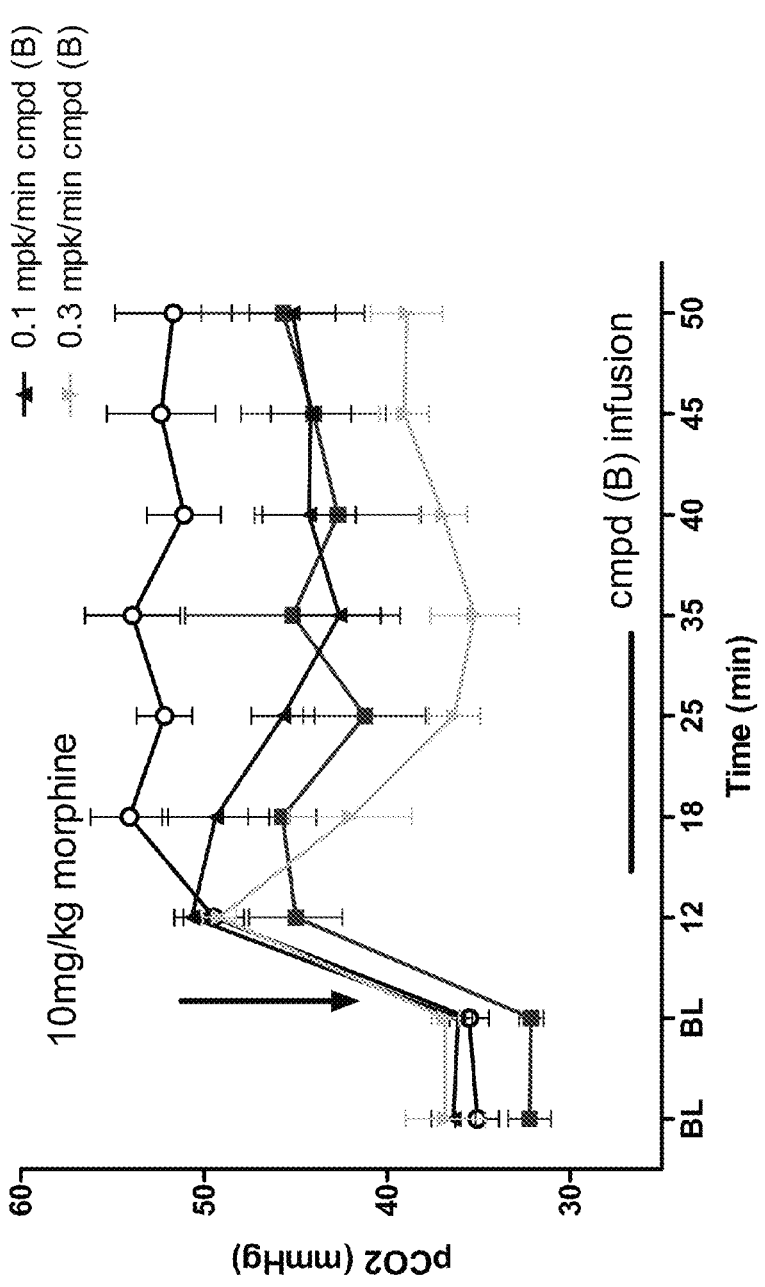

20% BCD
0.03 mpk/min cmpd (B)
0.1 mpk/min cmpd (B)
0.3 mpk/min cmpd (B)
10mg/kg morphine
cmpd (B) infusion
pCO2 (mmHg)
Time (min)
60
50
40
30
BL
BL
12
18
25
35
40
45
50

Fig. 8A

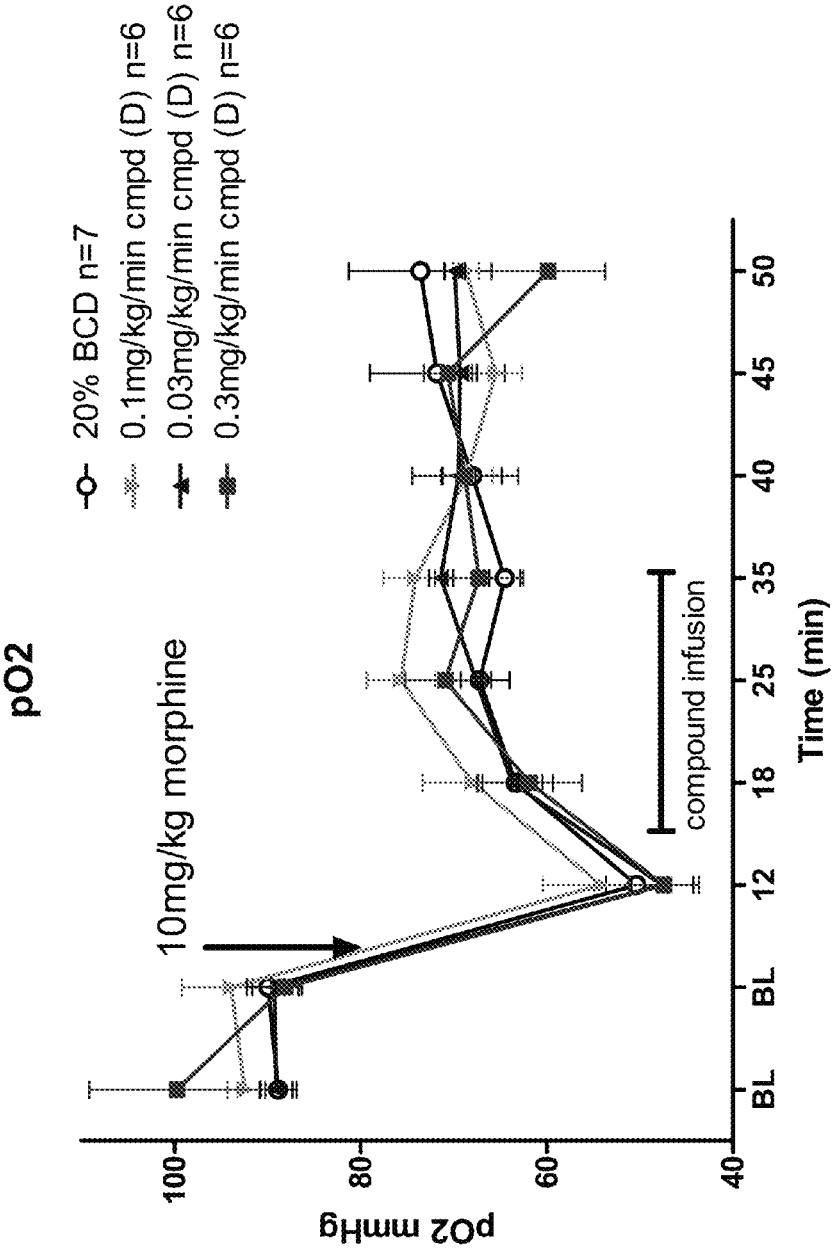

pO2
20% BCD n=7
0.1mg/kg/min cmpd (D) n=6
0.03mg/kg/min cmpd (D) n=6
0.3mg/kg/min cmpd (D) n=6
10mg/kg morphine
compound infusion
pO2 mmHg
100
80
60
40
BL
BL
12
18
25
35
40
45
50
Time (min)

Fig. 8B

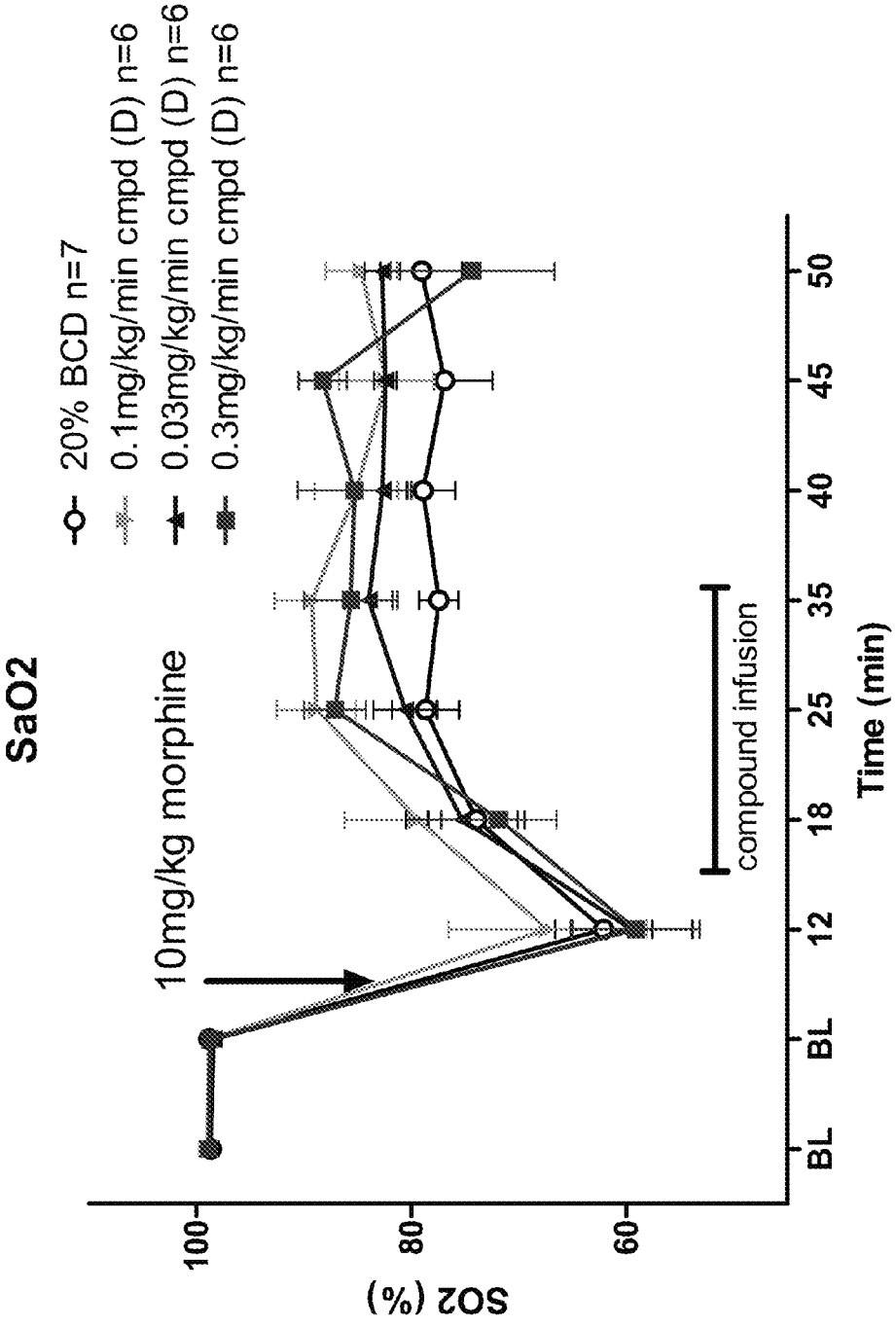

SaO2
20% BCD n=7
0.1mg/kg/min cmpd (D) n=6
0.03mg/kg/min cmpd (D) n=6
0.3mg/kg/min cmpd (D) n=6
10mg/kg morphine
compound infusion
SO2 (%)
100
80
60
BL
BL
12
18
25
35
40
45
50
Time (min)

Fig. 8C

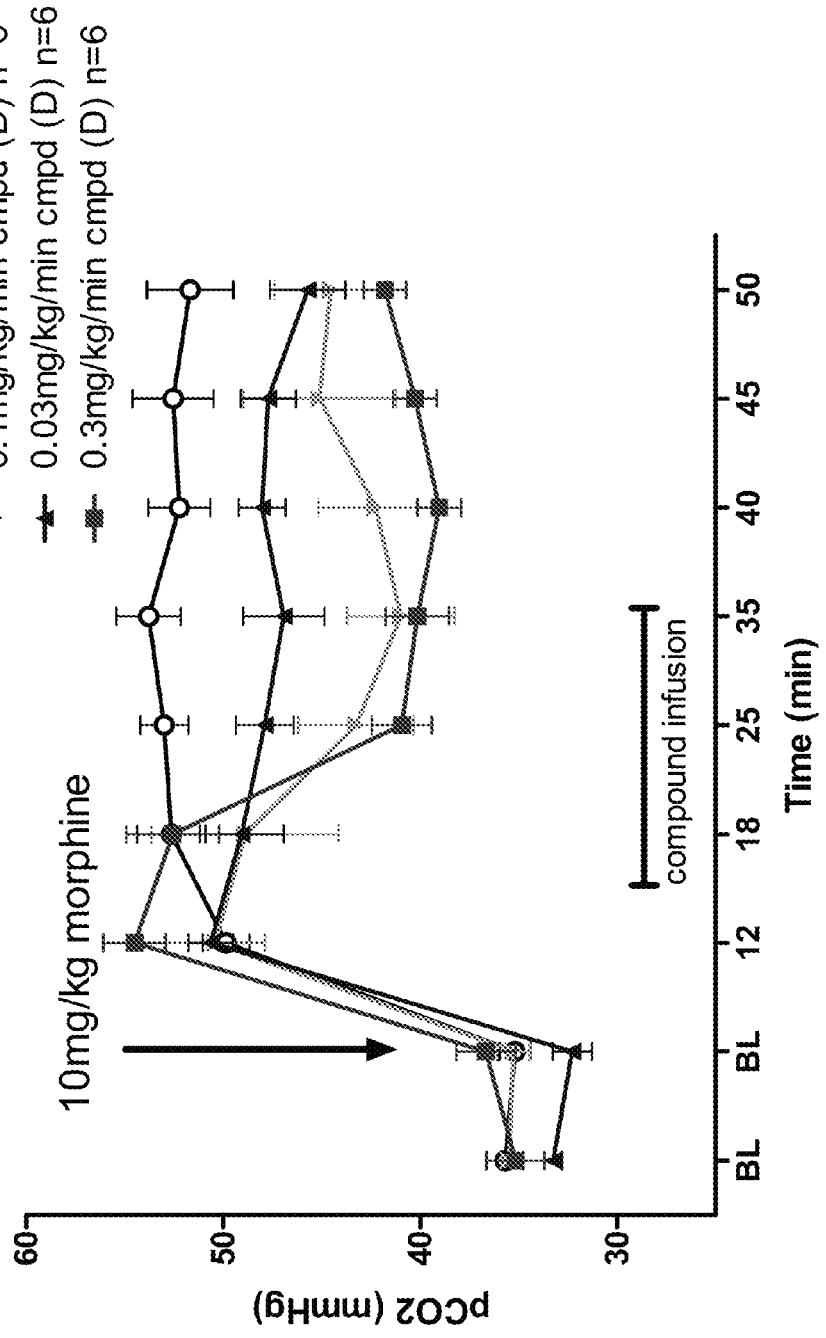

pCO2
20% BCD n=7
0.1mg/kg/min cmpd (D) n=6
0.03mg/kg/min cmpd (D) n=6
0.3mg/kg/min cmpd (D) n=6
10mg/kg morphine
compound infusion
pCO2 (mmHg)
60
50
40
30
BL
BL
12
18
25
35
40
45
50
Time (min)

Fig. 8D

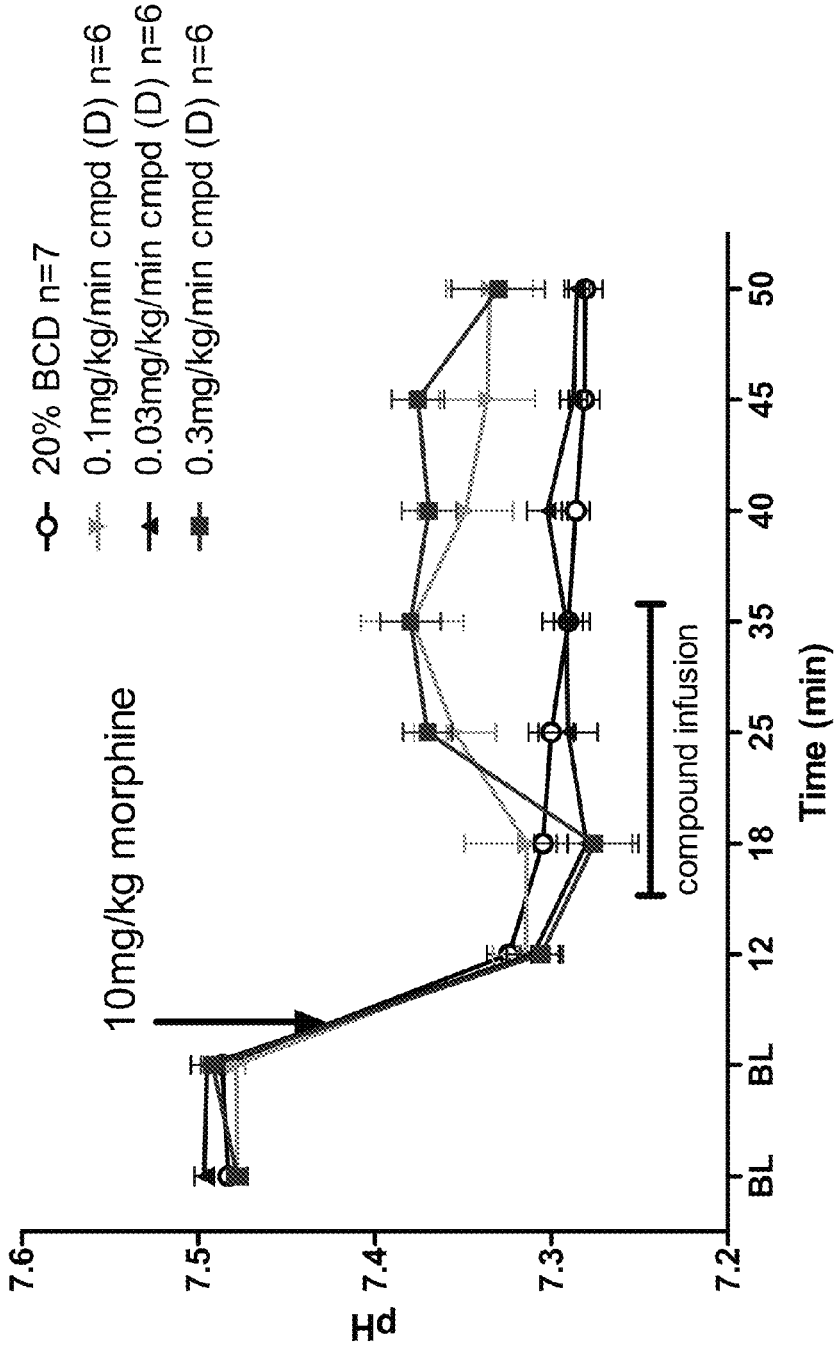
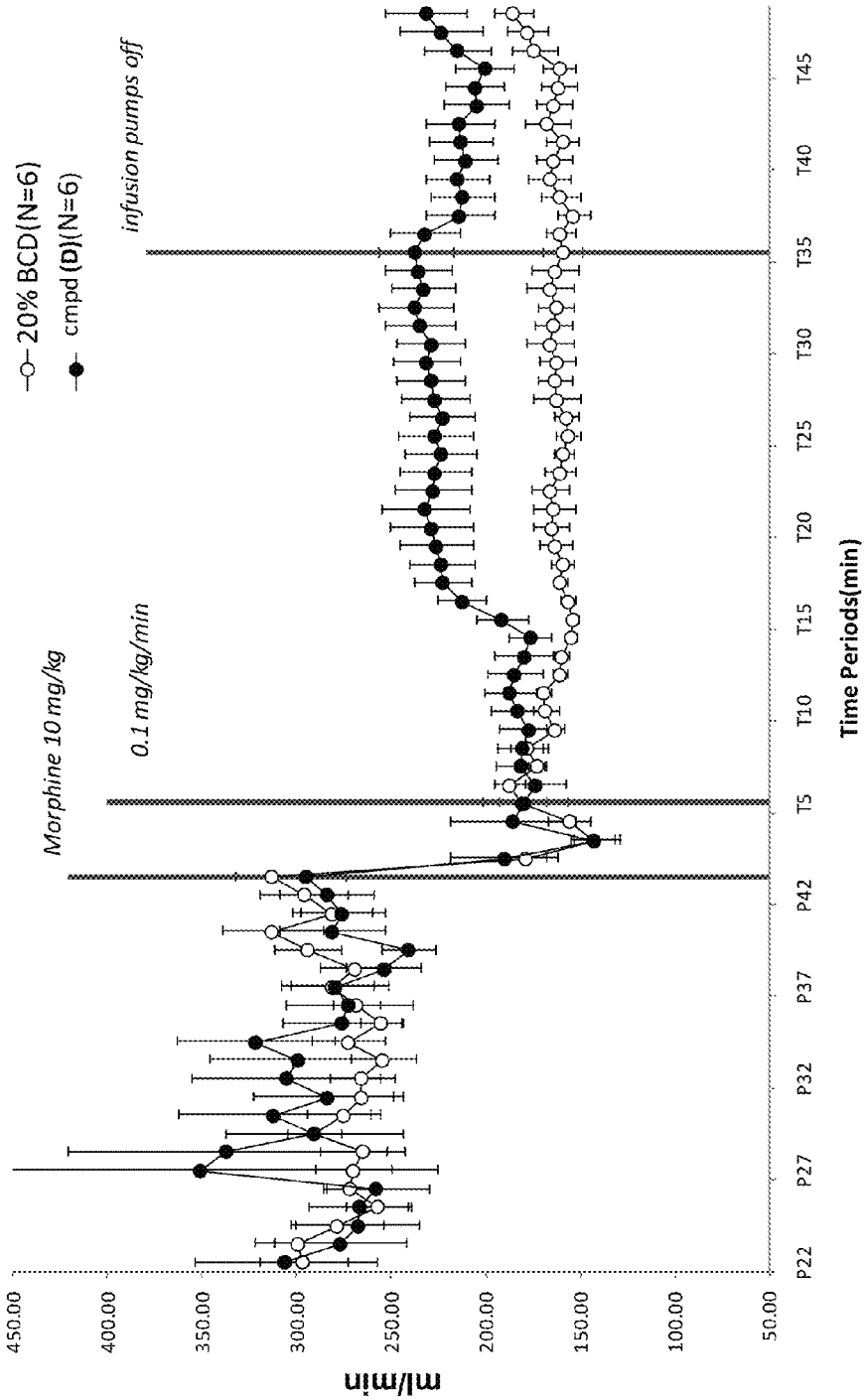

pH
20% BCD n=7
0.1mg/kg/min cmpd (D) n=6
0.03mg/kg/min cmpd (D) n=6
0.3mg/kg/min cmpd (D) n=6
10mg/kg morphine
compound infusion
pH
Time (min)
7.6
7.5
7.4
7.3
7.2
BL
BL
12
18
25
35
40
45
50

Fig. 9


20% BCD(N=6)
cmpd (D)(N=6)
Morphine 10 mg/kg
0.1 mg/kg/min
infusion pumps off
ml/min
450.00
400.00
350.00
300.00
250.00
200.00
150.00
100.00
50.00
P22
P27
P32
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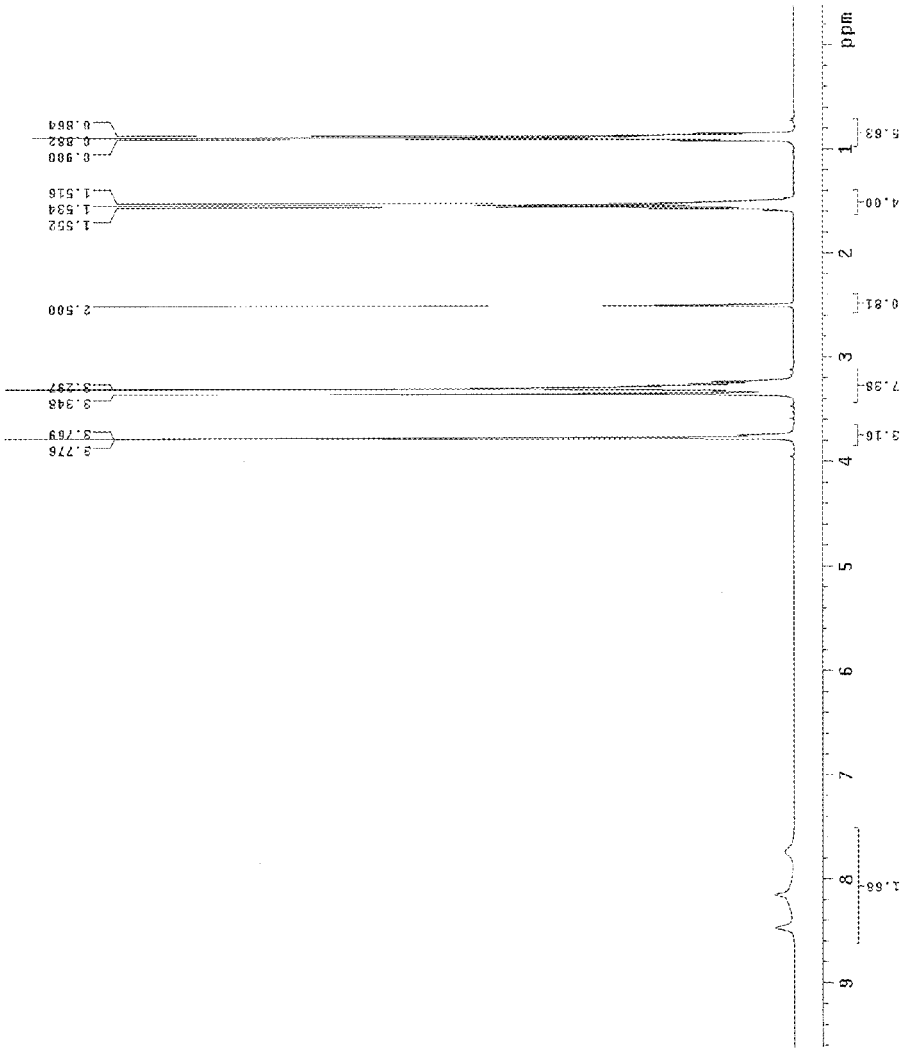
P42
T5
T10
T15
T20
T25
T30
T35
T40
T45
Time Periods(min)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0.980
1.516
1.534
1.552
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5.83
4.00
0.81
7.38
3.16
1.66

Fig. 10

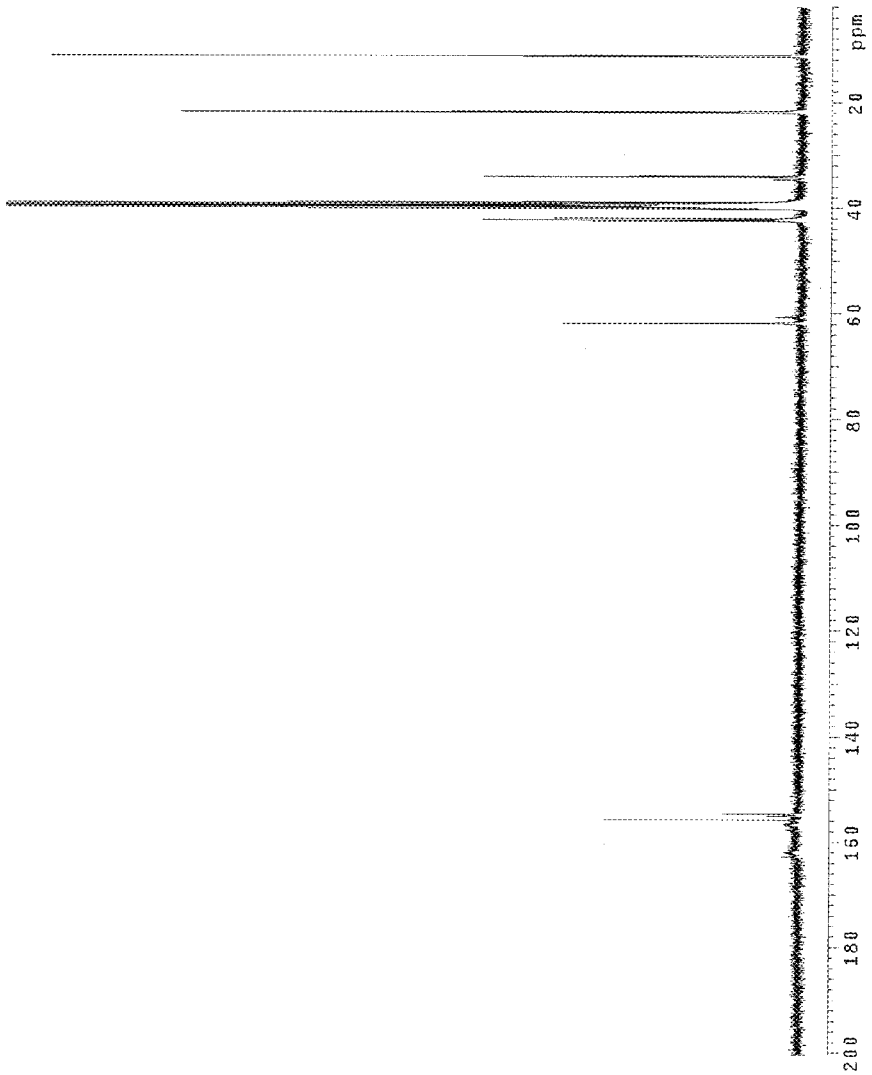
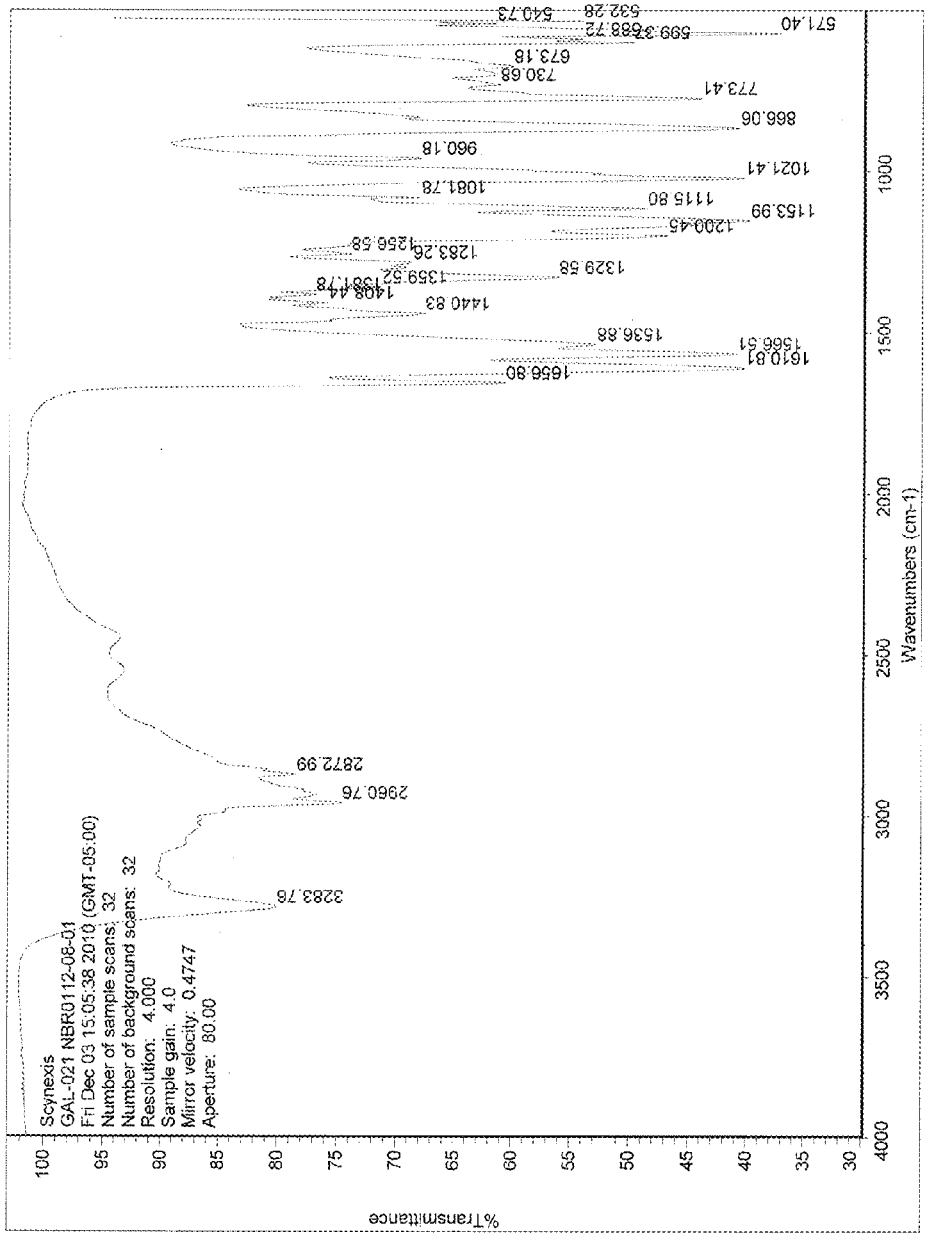


Fig. 11

Fig. 12


%Transmittance
Wavenumbers (cm-1)
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Number of background scans: 32
Resolution: 4.000
Sample gain: 4.0
Mirror velocity: 0.4747
Aperture: 80.00
3283.76
2960.76
2872.99
1656.80
1610.81
1566.51
1536.86
1440.83
1408.44
1381.78
1359.52
1329.58
1283.26
1256.58
1200.45
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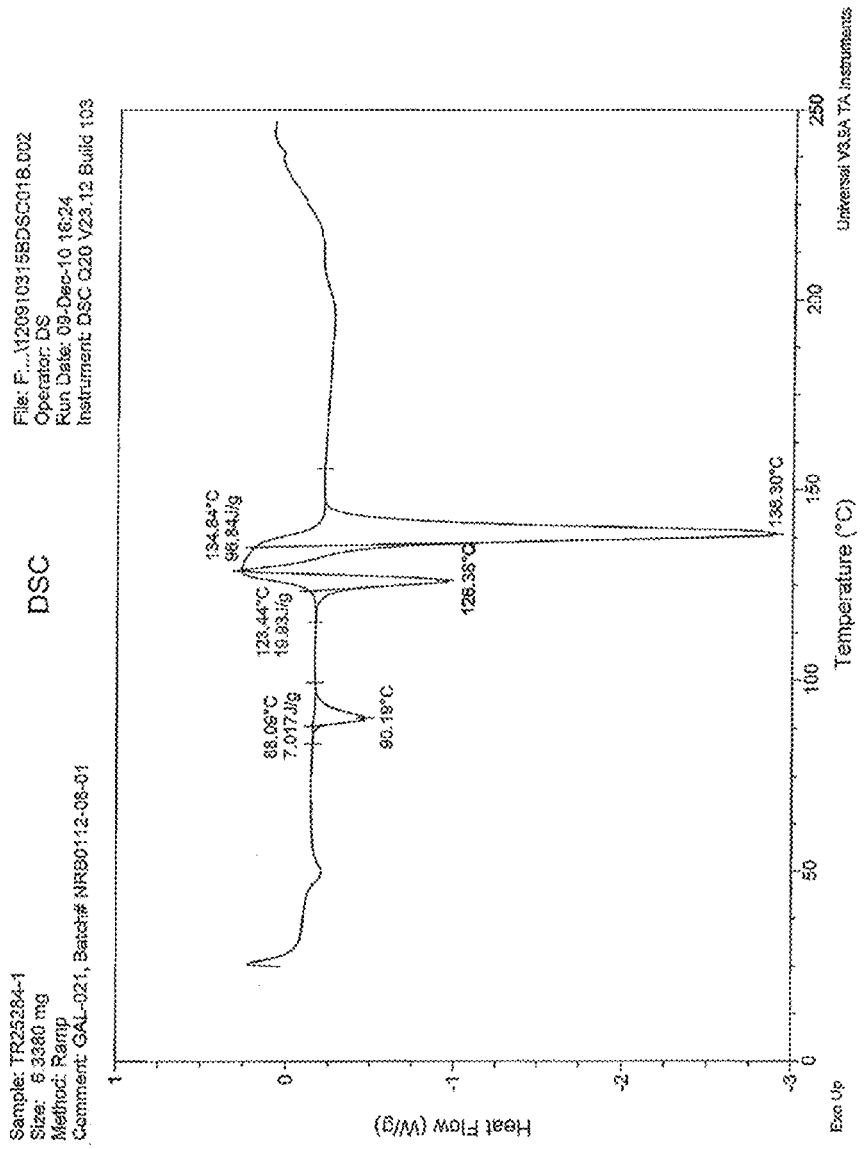
571.40
540.73
532.28

Fig. 13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Sample: TR25284-1
Size: 6.3380 mg
Method: Ramp
Comment: GAL-021, Batch# NRB0112-08-01
File: F:...\120910315BDSC018.002
Operator: DS
Run Date: 09-Dec-10 16:24
Instrument: DSC Q20 V24.12 Build 103
134.84°C
96.84 J/g
128.44°C
19.83 J/g
88.06°C
7.017 J/g
86.19°C
126.38°C
138.30°C
Heat Flow (W/g)
Temperature (°C)
Exo Up
Universal V3.5A TA Instruments

Fig. 14

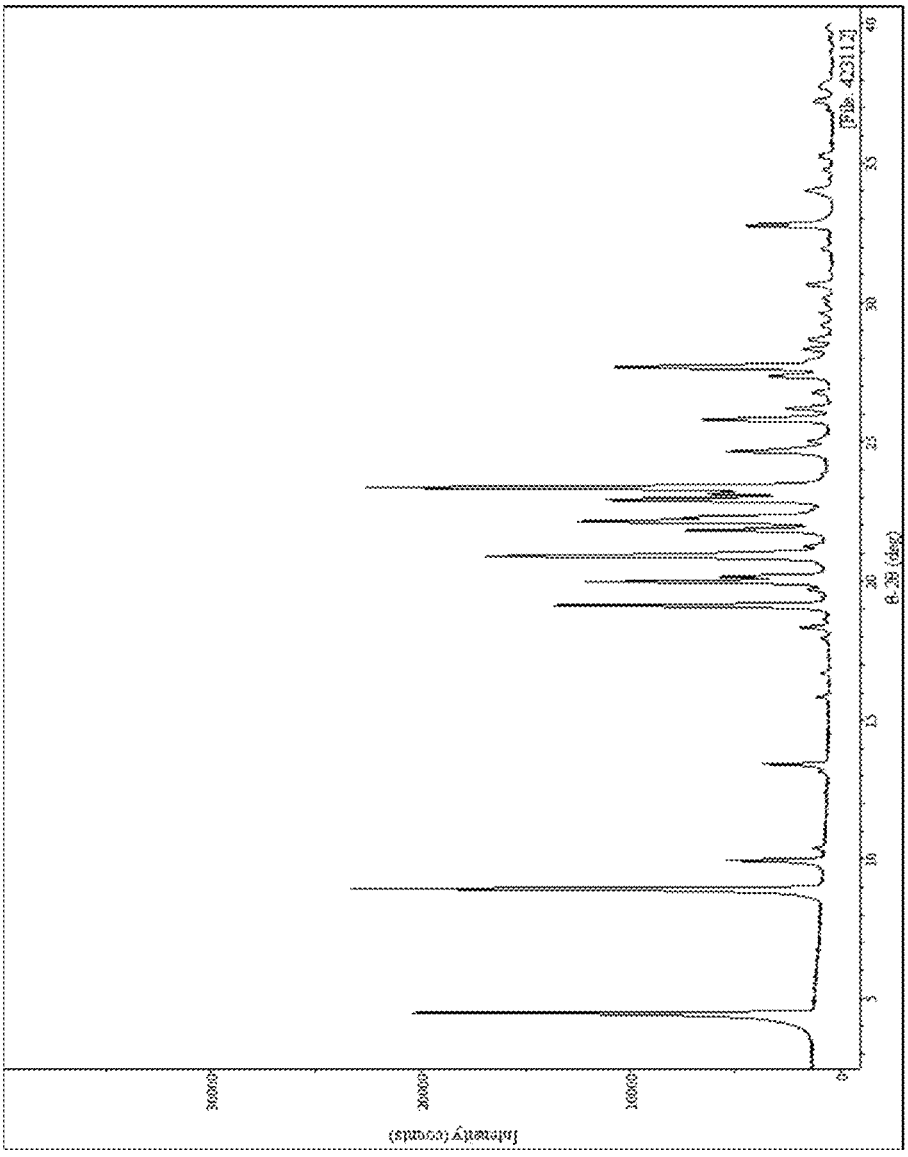

Intensity (counts)
300000
200000
100000
0
5
10
15
20
25
30
35
40
θ-2θ (deg)
File: 423113

Fig. 15

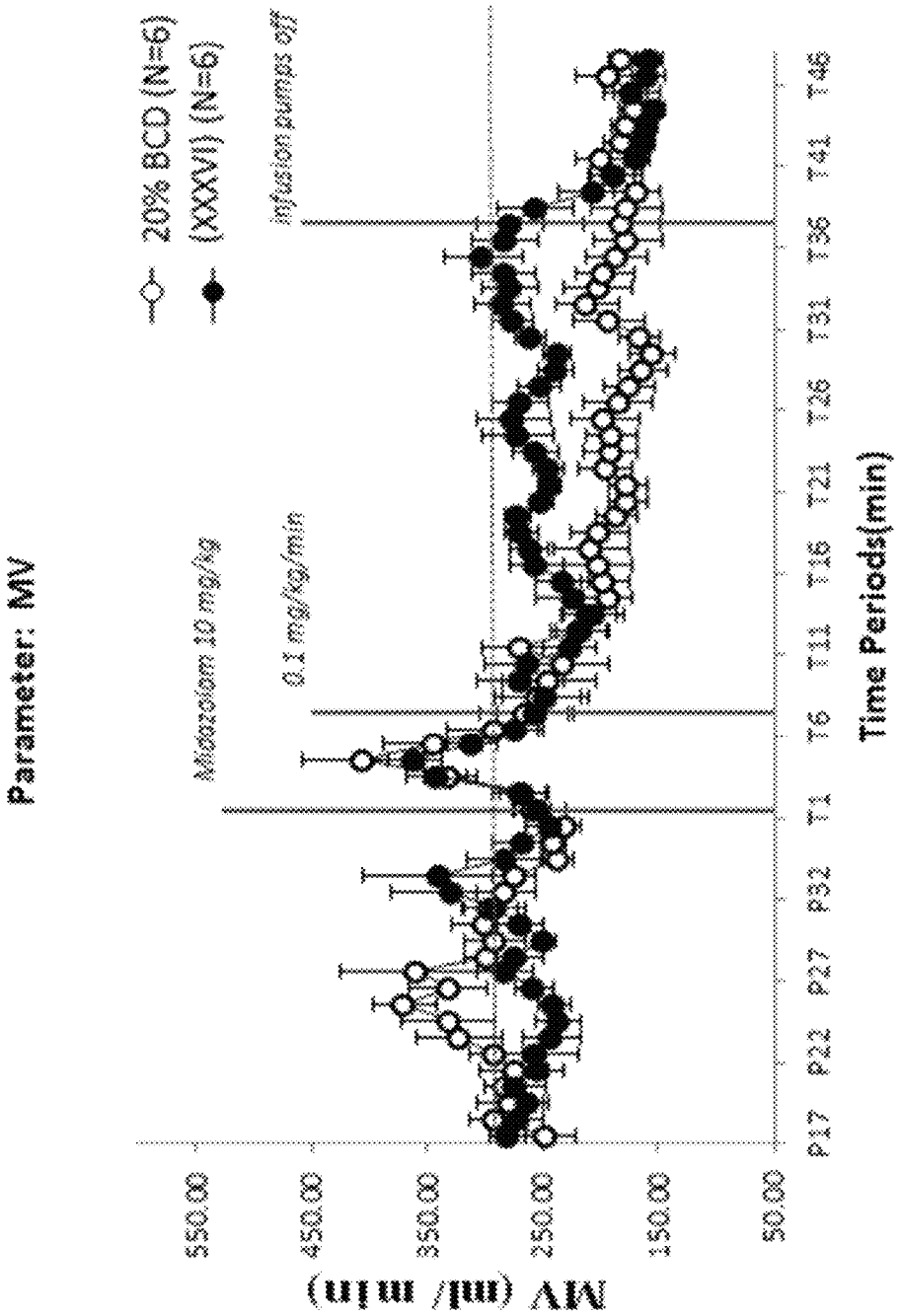

Parameter: MV
20% BCD (N=6)
(XXVII) (N=6)
Midazolam 10 mg/kg
0.1 mg/kg/min
Infusion pumps off
MV (ml/ min)
550.00
450.00
350.00
250.00
150.00
50.00
P17 P22 P27 P32 T1 T6 T11 T16 T21 T26 T31 T36 T41 T46
Time Periods(min)

Fig. 16

Parameter: TV

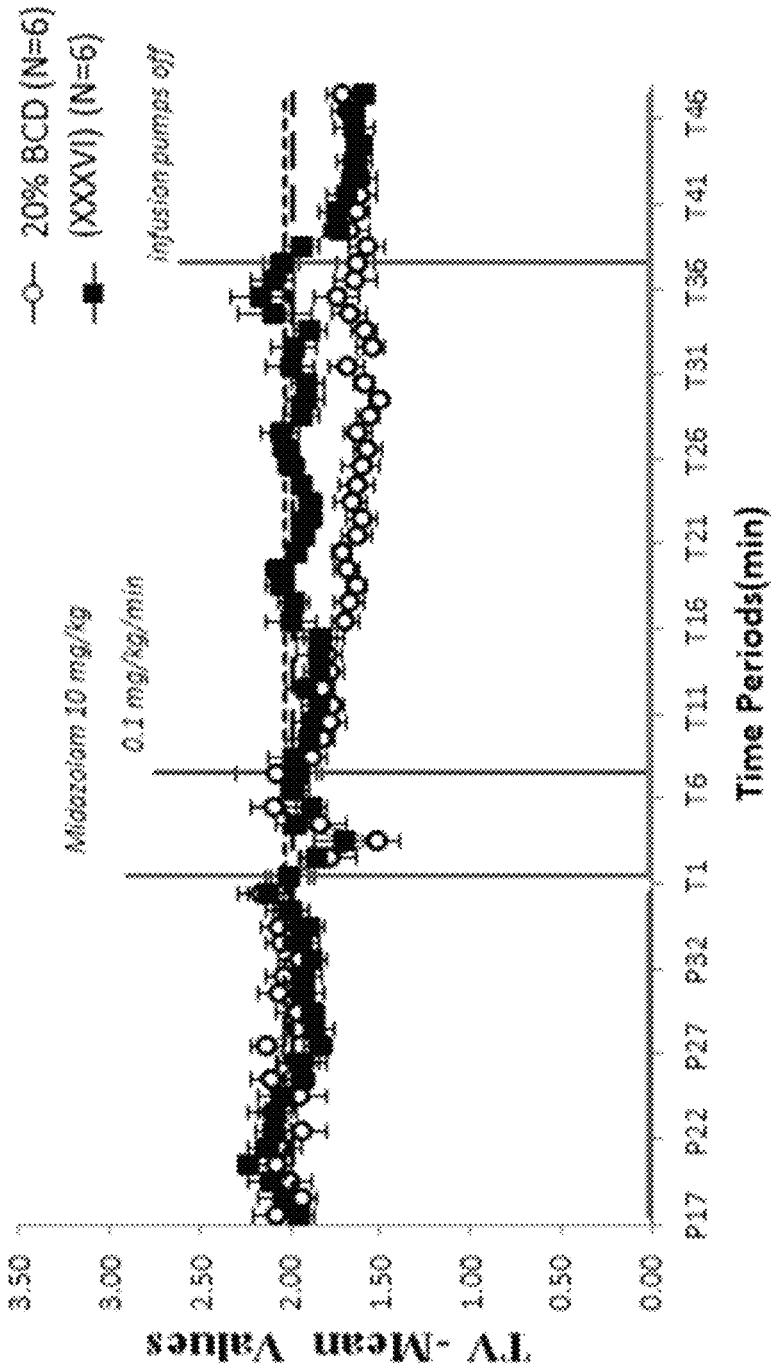


Fig. 17

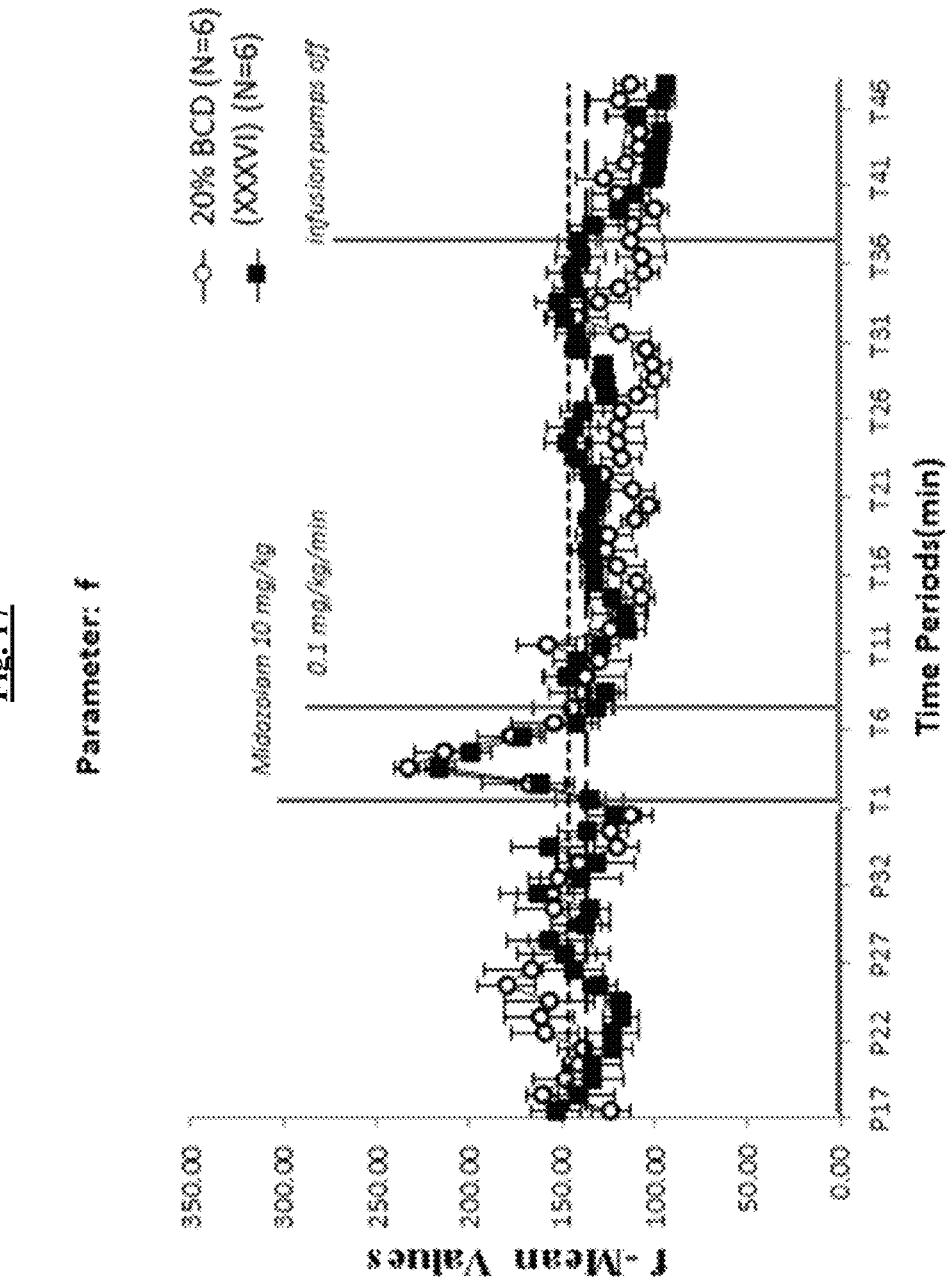

Parameter: f
20% BCD (N=6)
(XXVI) (N=6)
Midazolam 10 mg/kg
0.1 mg/kg/min
Infusion pumps off
f - Mean Values
350.00
300.00
250.00
200.00
150.00
100.00
50.00
0.00
P17 P22 P27 P32 T1 T6 T11 T16 T21 T26 T31 T36 T41 T46
Time Periods(min)

Fig. 18

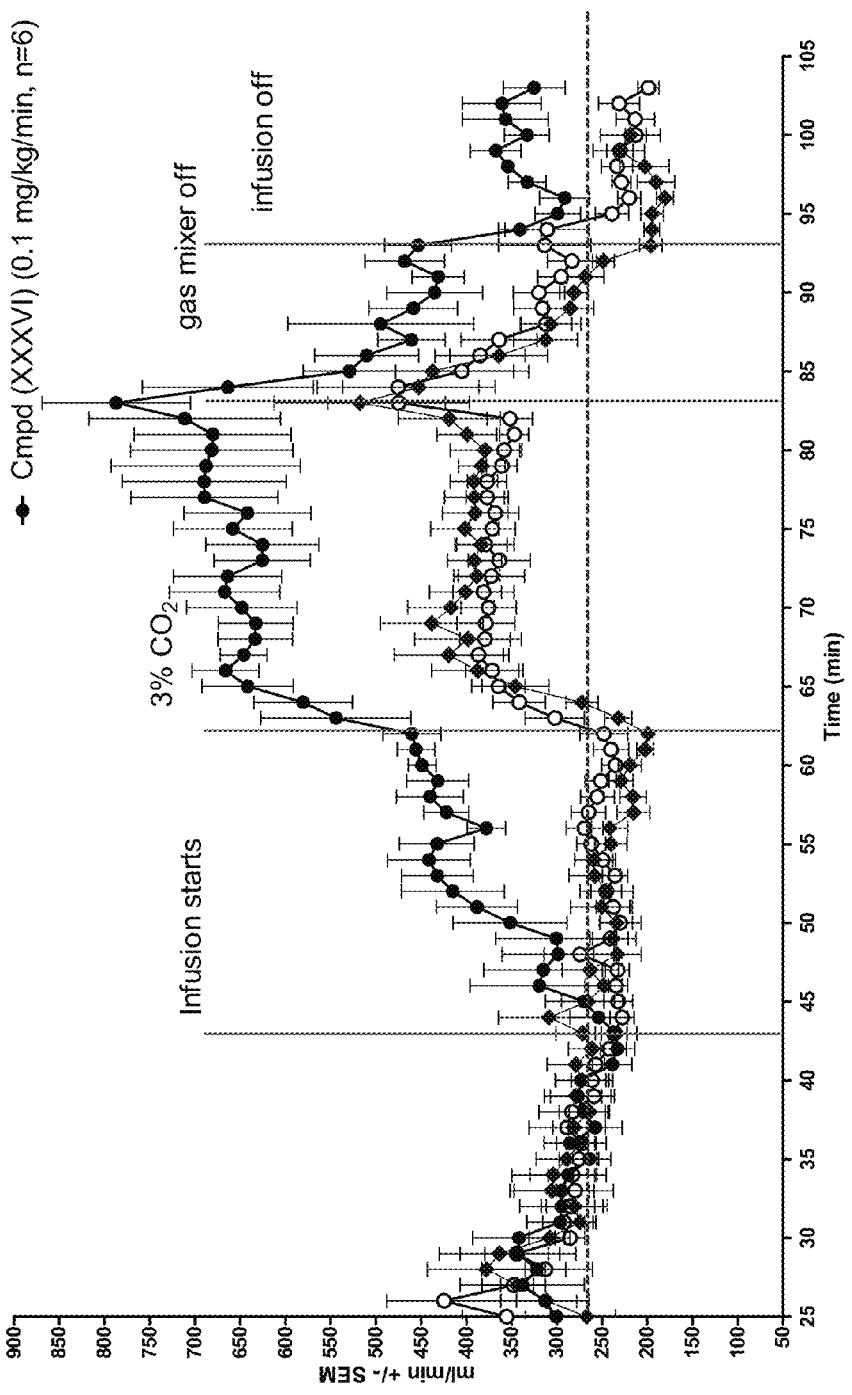

20% BCD (n=12)
Cmpd (XXXVI) (0.03 mg/kg/min, n=6)
Cmpd (XXXVI) (0.1 mg/kg/min, n=6)
Infusion starts
3% CO_2
gas mixer off
infusion off
ml/min +/- SEM
Time (min)

Fig. 19A

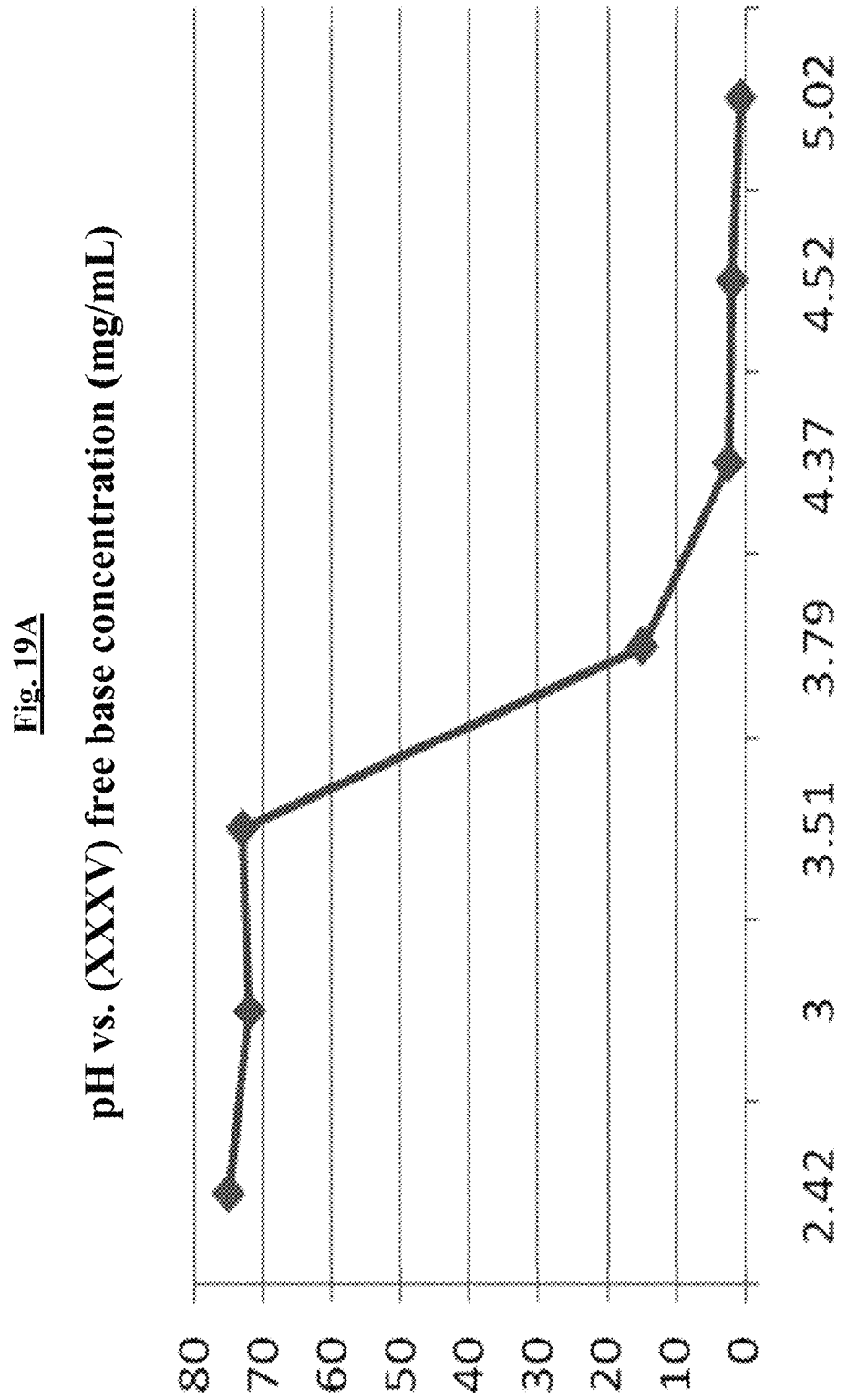

pH vs. (XXXV) free base concentration (mg/mL)
80
70
60
50
40
30
20
10
0
2.42
3
3.51
3.79
4.37
4.52
5.02

Fig. 19B

pH solubility Profile of (XXXV)

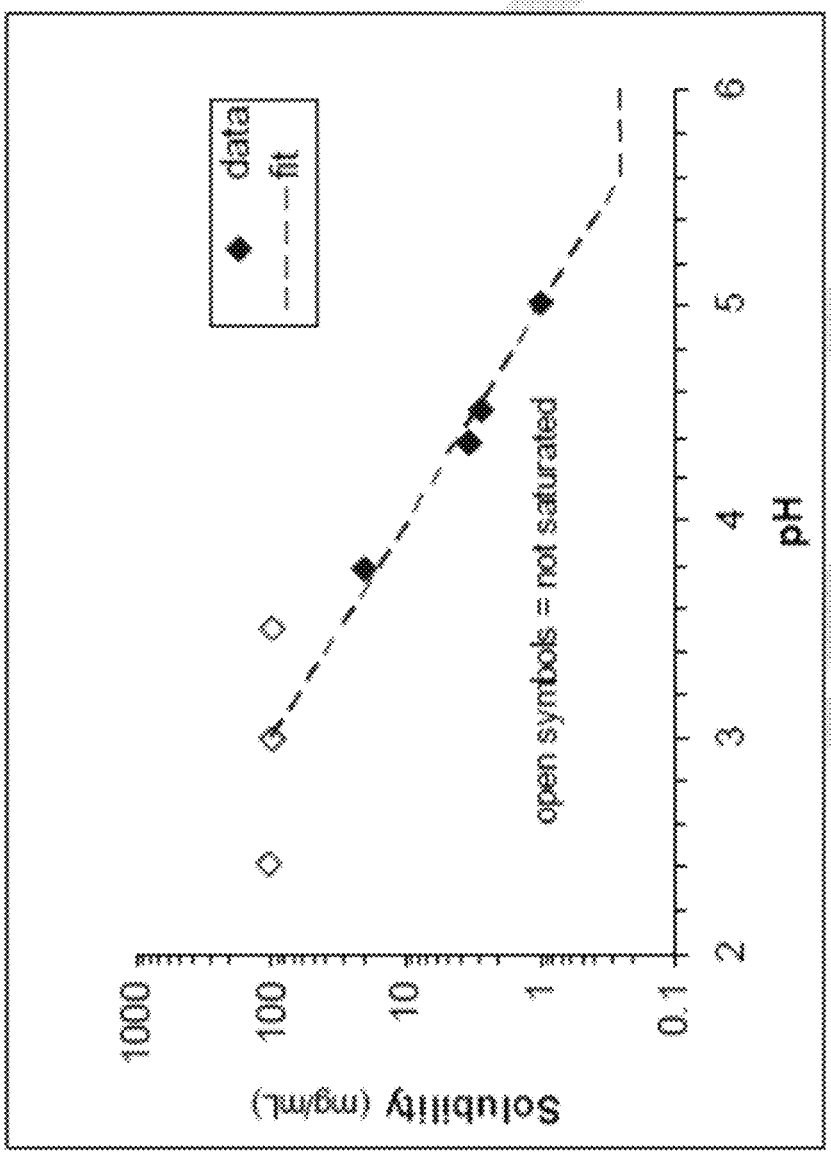


Fig. 20A

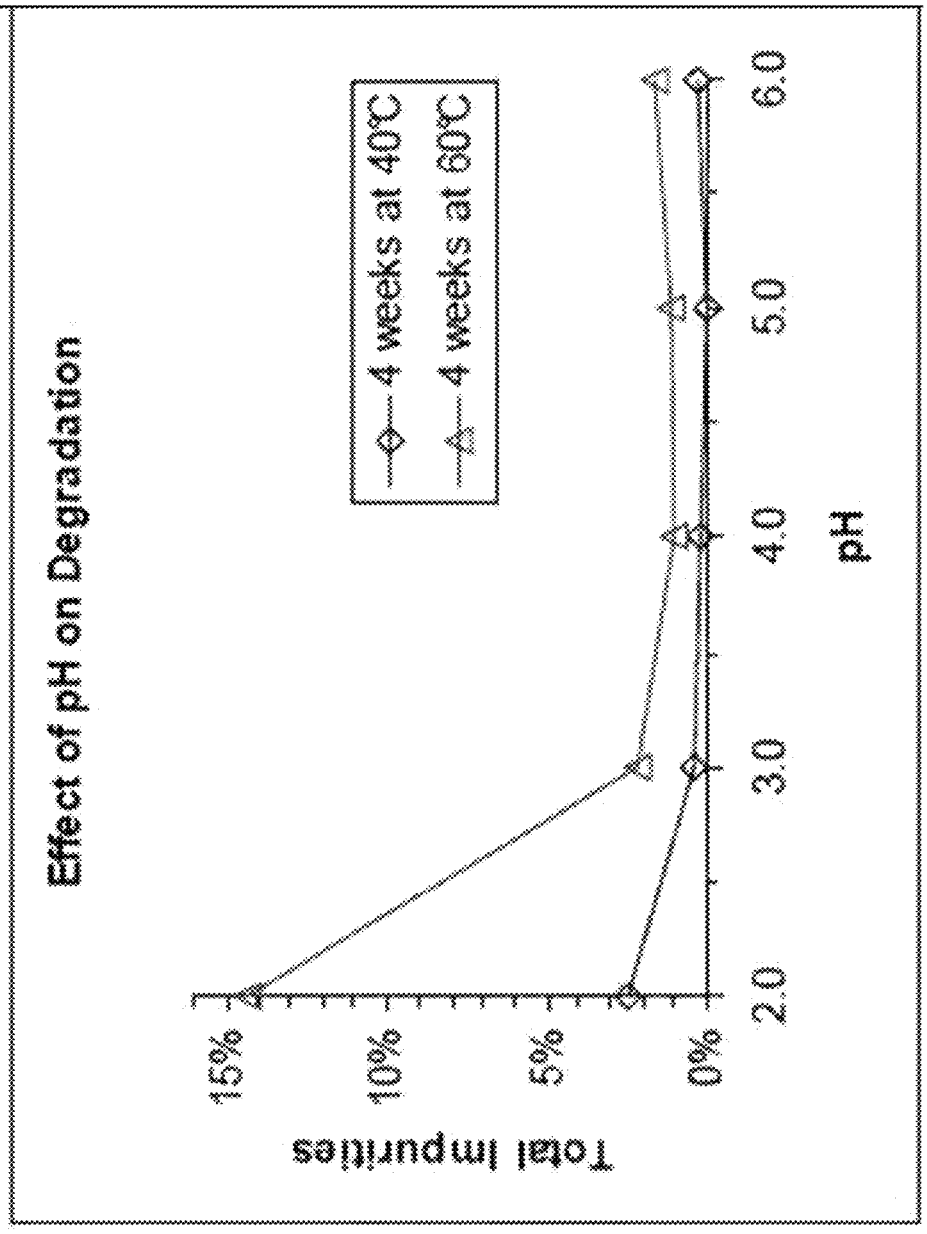

Effect of pH on Degradation
4 weeks at 40°C
4 weeks at 80°C
Total Impurities
15%
10%
5%
0%
2.0
3.0
4.0
5.0
6.0
pH

Fig. 20B

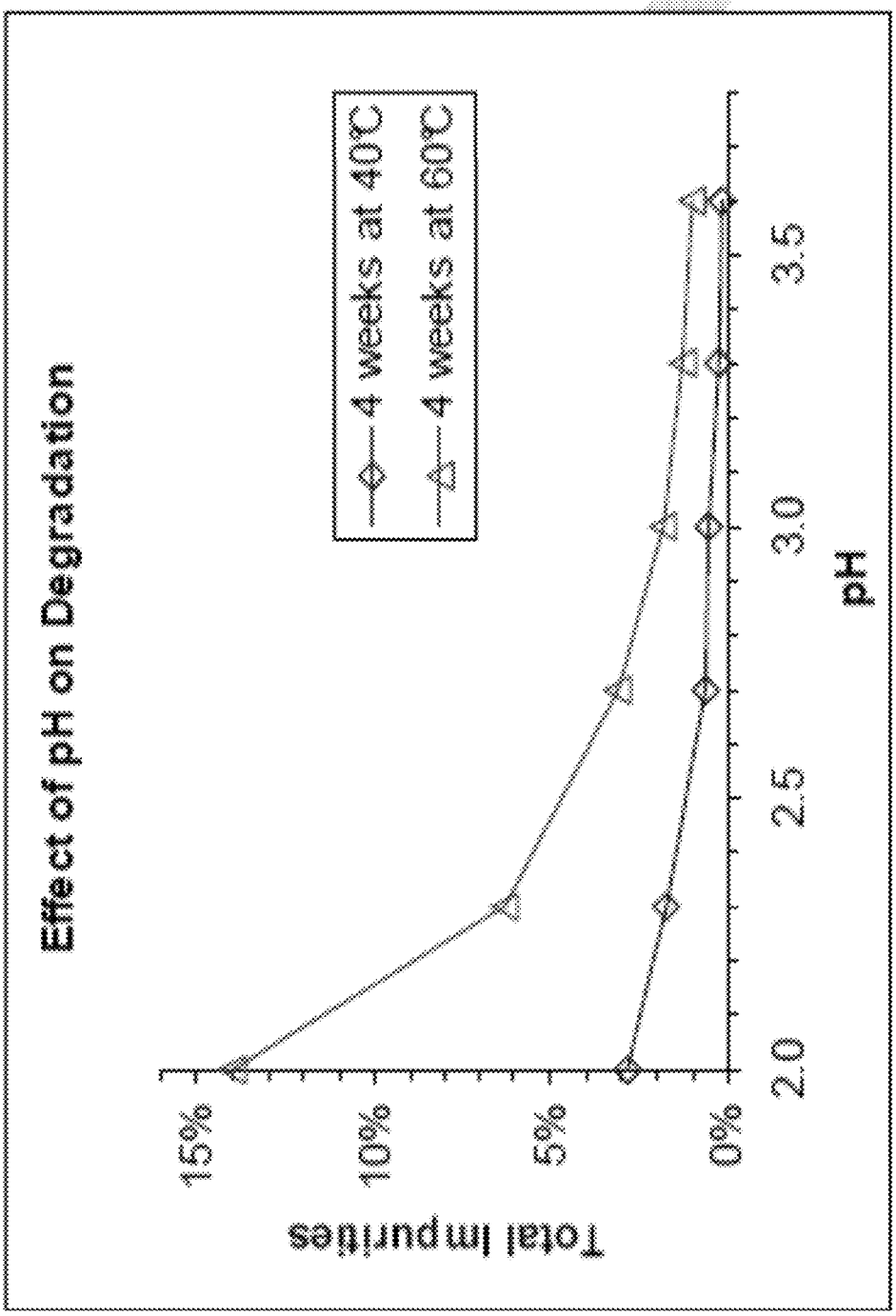

Effect of pH on Degradation
4 weeks at 40°C
4 weeks at 60°C
Total Impurities
15%
10%
5%
0%
2.0
2.5
3.0
3.5
pH

Fig. 21

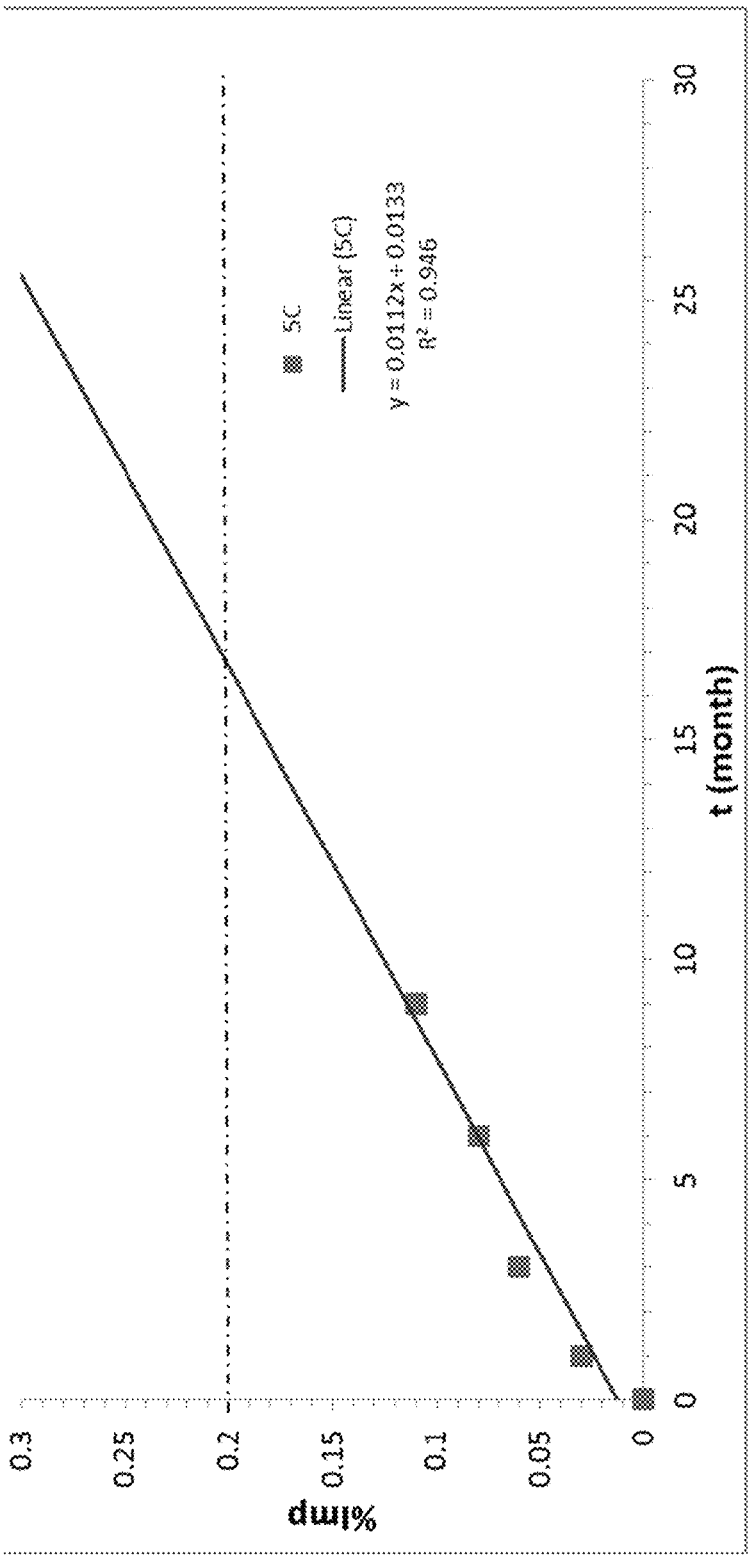

Formation Kinetics of (CLXXVIII) impurity (RRT0.67)
%Imp
t (month)
0.3
0.25
0.2
0.15
0.1
0.05
0
0
5
10
15
20
25
30
5C
Linear (5C)
y = 0.0112x + 0.0133
R² = 0.946

Fig. 22

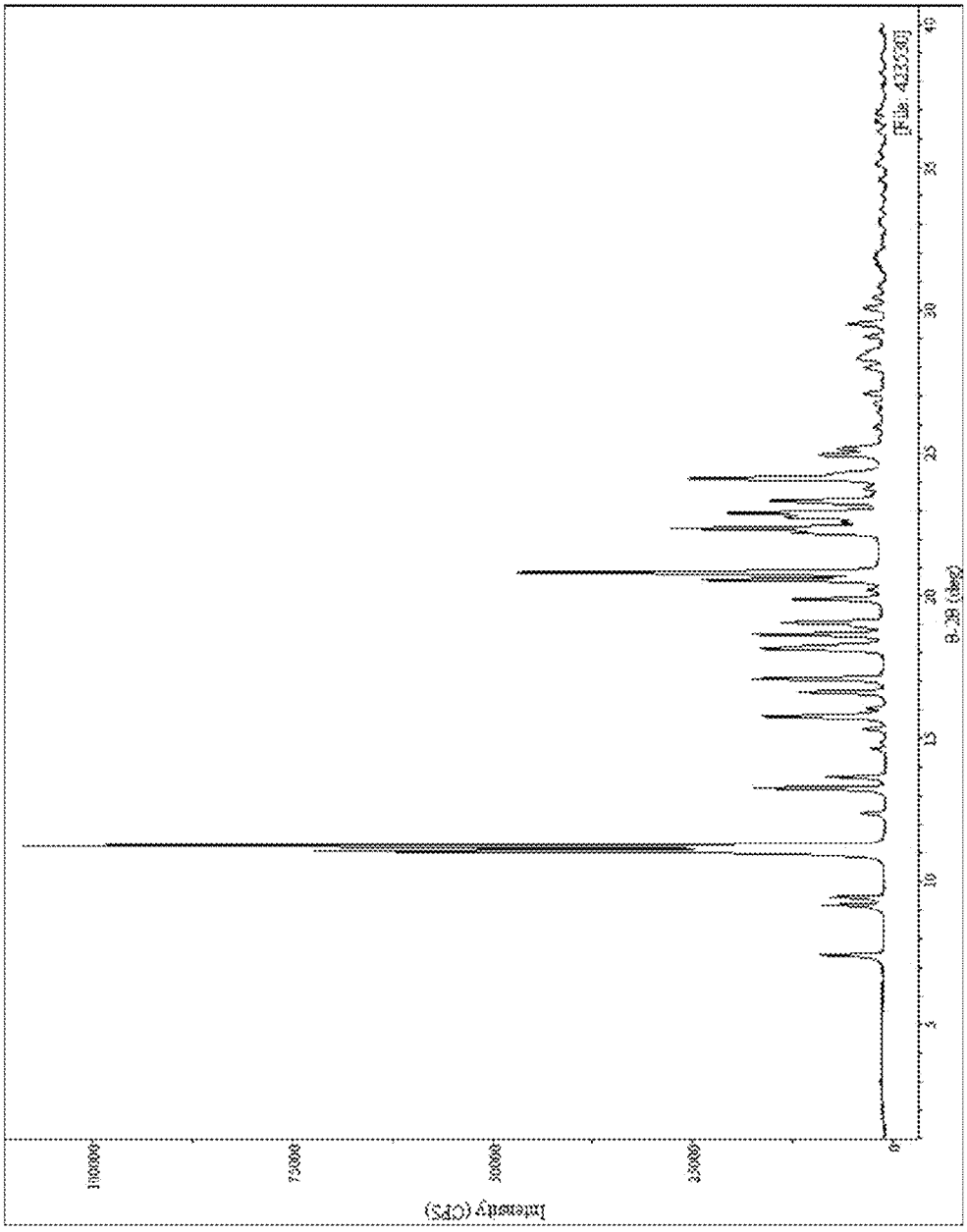

Intensity (CPS)
150000
75000
50000
25000
0
5
10
15
20
25
30
35
40
2-Theta (deg)
[File: 432520]

Fig. 23

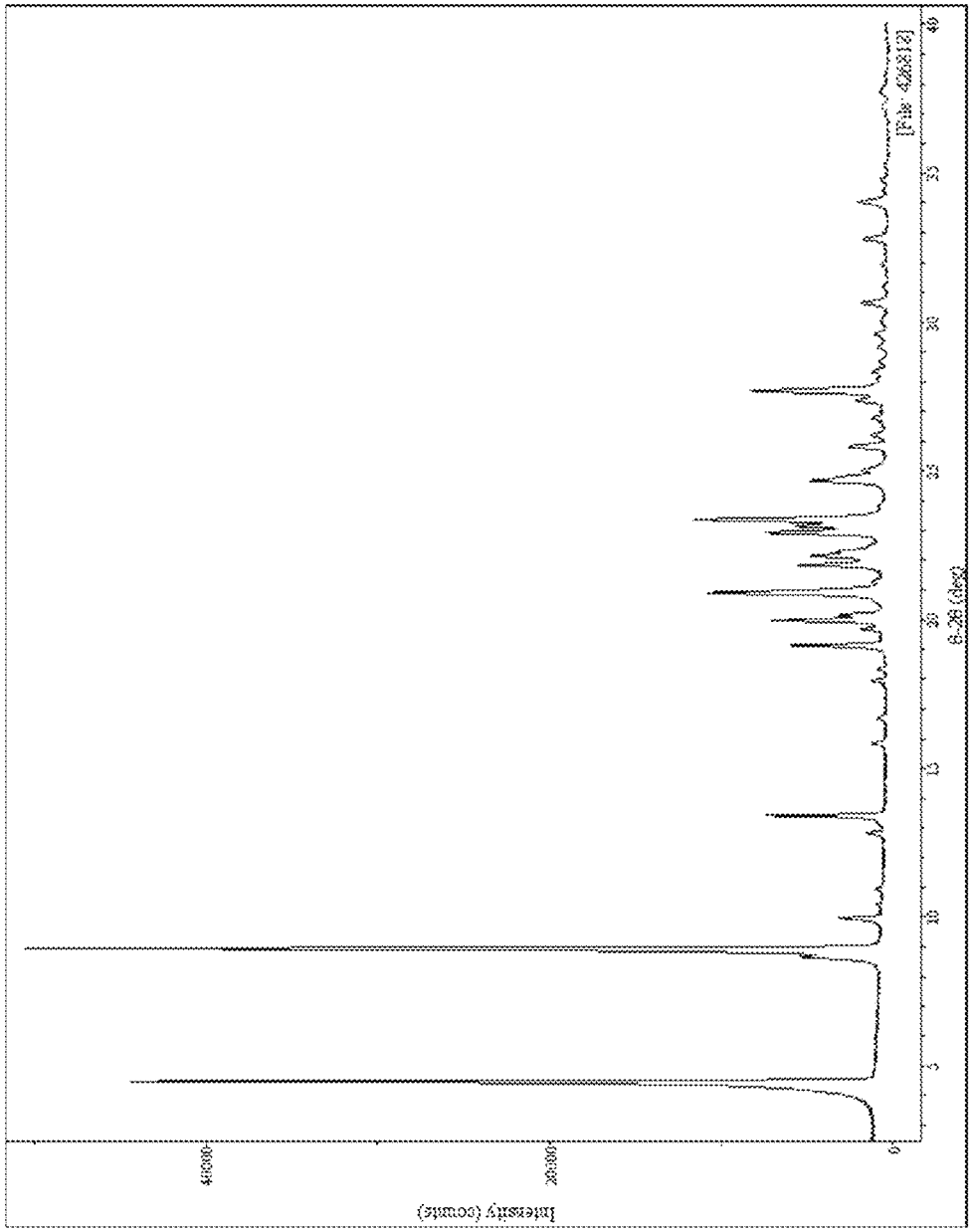

Intensity (counts)
40000
20000
0
5
10
15
20
25
30
35
40
[File: 426812]
θ-2θ (deg)

Fig. 24

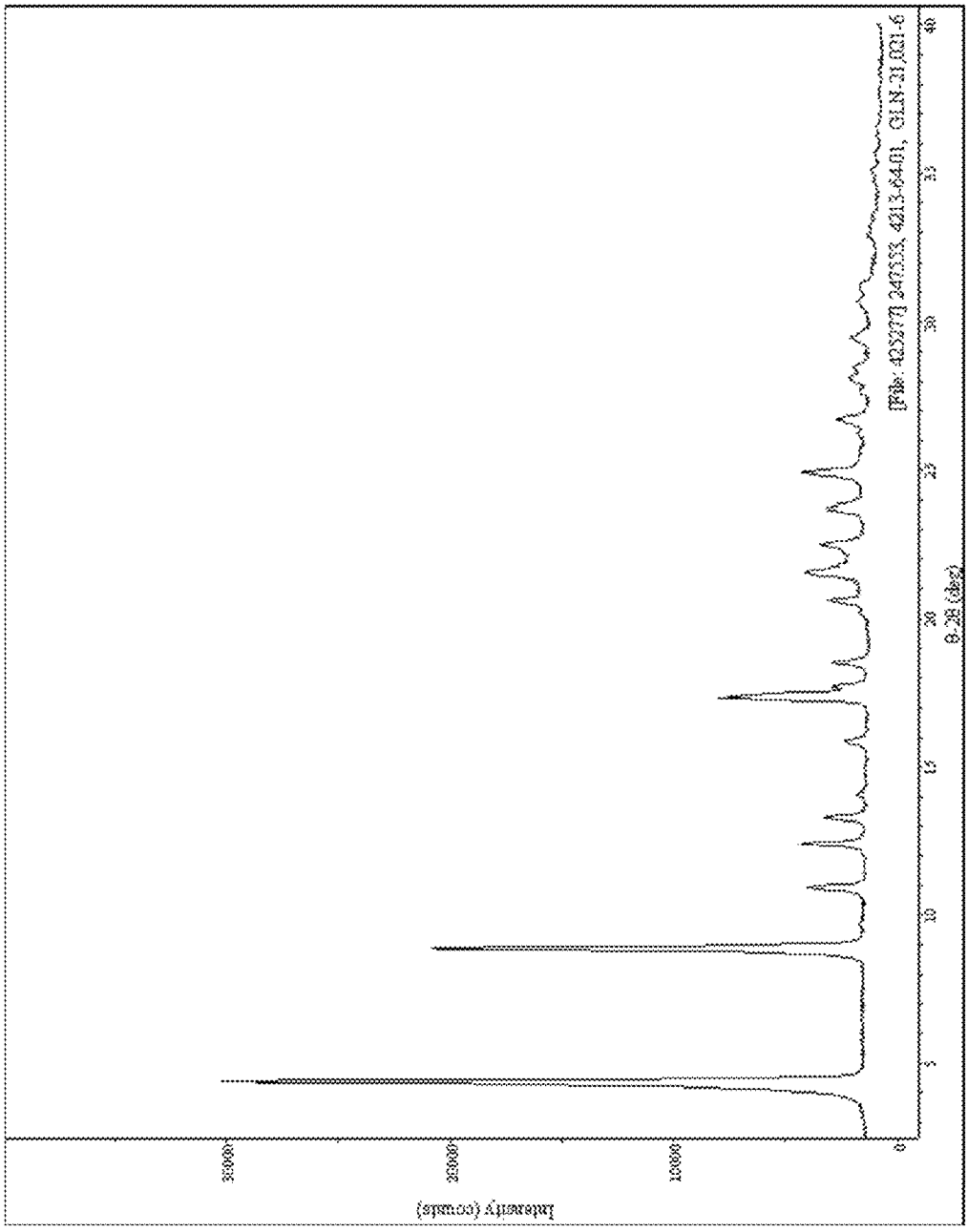

Intensity (counts)
30000
20000
10000
0
5
10
15
20
25
30
35
40
2-Theta (deg)
[File: 425277] 247533, 4213-64401, C1.N-31.021-6

Fig. 25

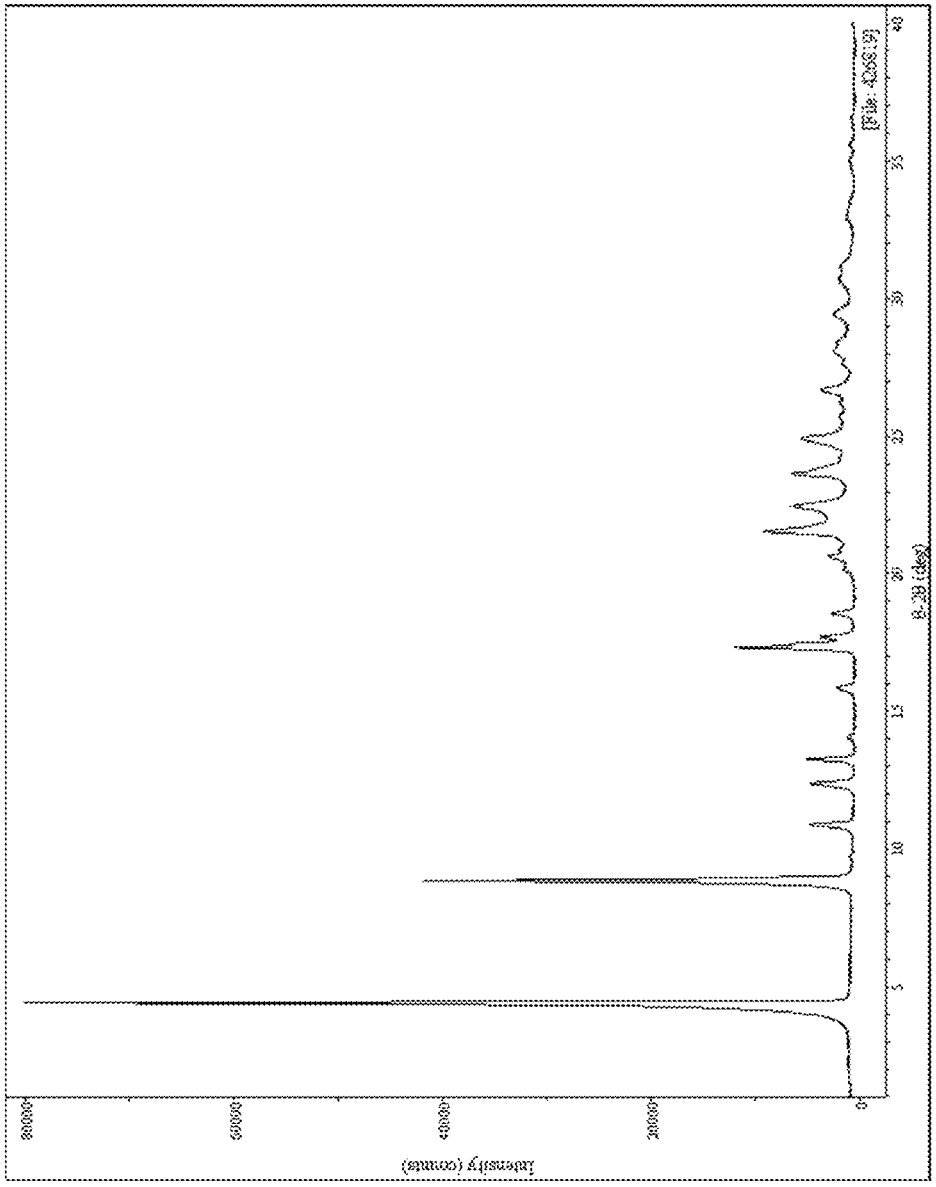

Intensity (counts)
60000
40000
20000
0
5
10
15
20
25
30
35
40
θ-2θ (deg)
[File: 4268.rd]

Fig. 26

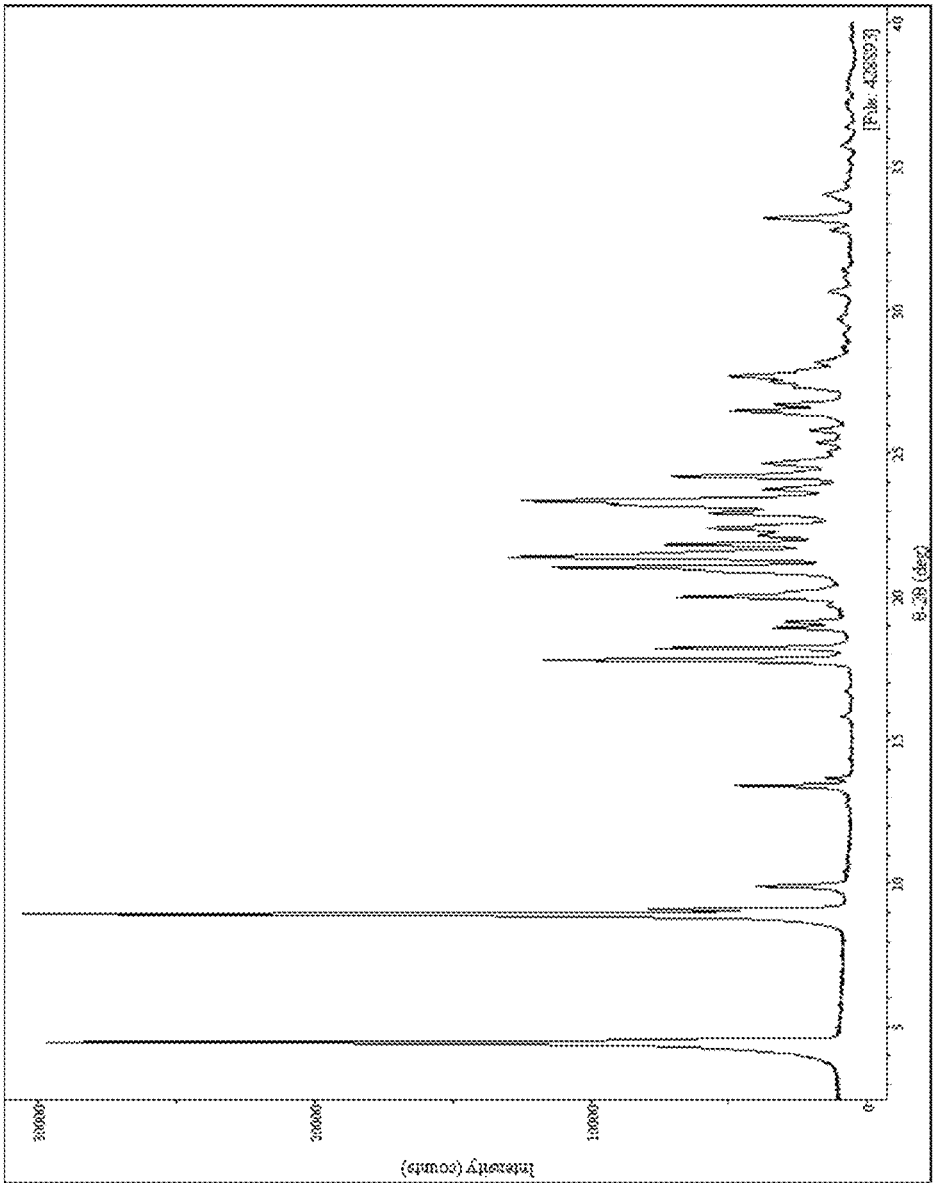


Fig. 27

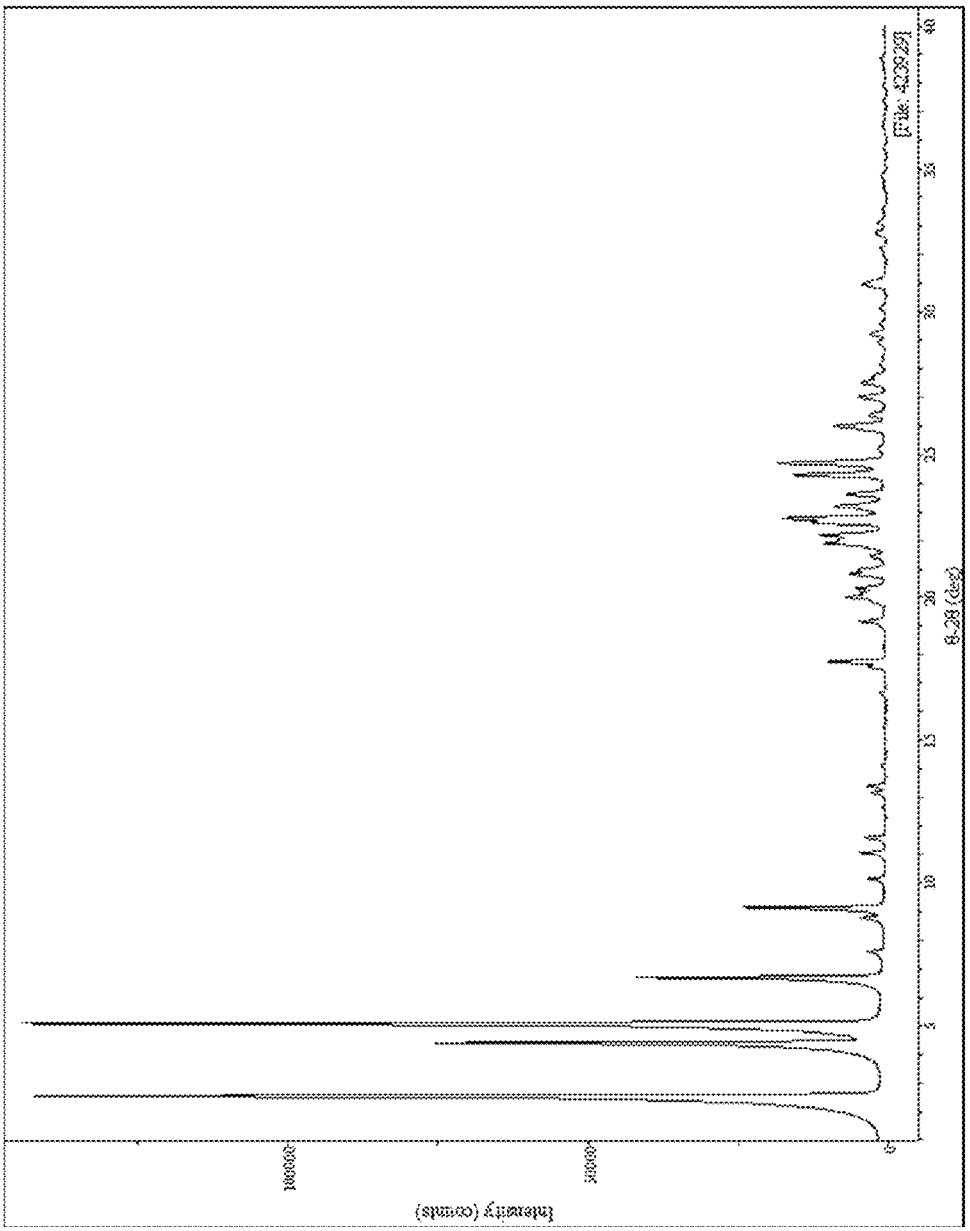

Intensity (counts)
100000
50000
0
5
10
15
20
25
30
35
40
θ-2θ (deg)
File: 4239.25]

Fig. 28

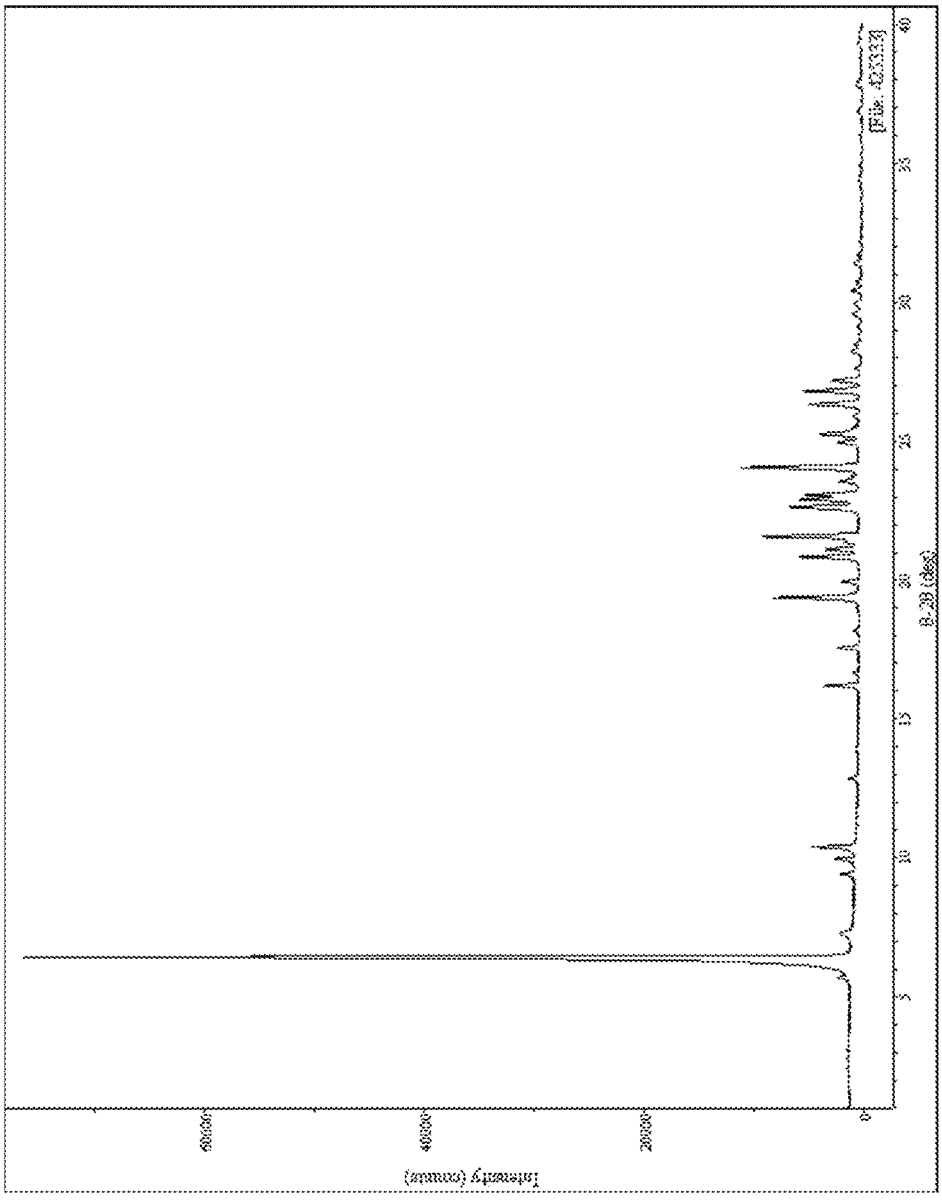

Intensity (counts)
600000
400000
200000
0
5
10
15
20
25
30
35
40
[File: 425333]
θ-2θ (deg)

Fig. 29

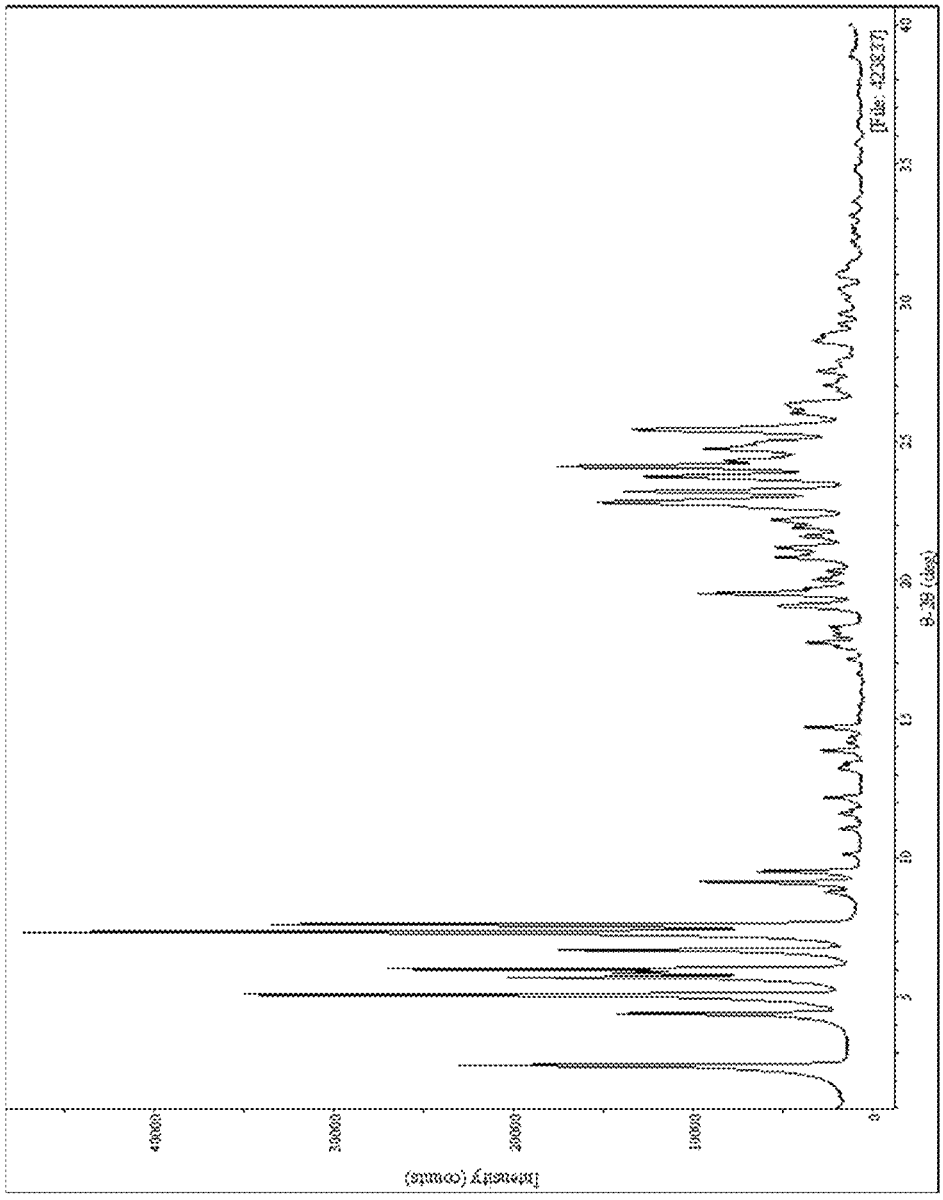

Intensity (counts)
40000
30000
20000
10000
0
5
10
15
20
25
30
35
40
2-Theta (deg)
[File: 4230377]

Fig. 30

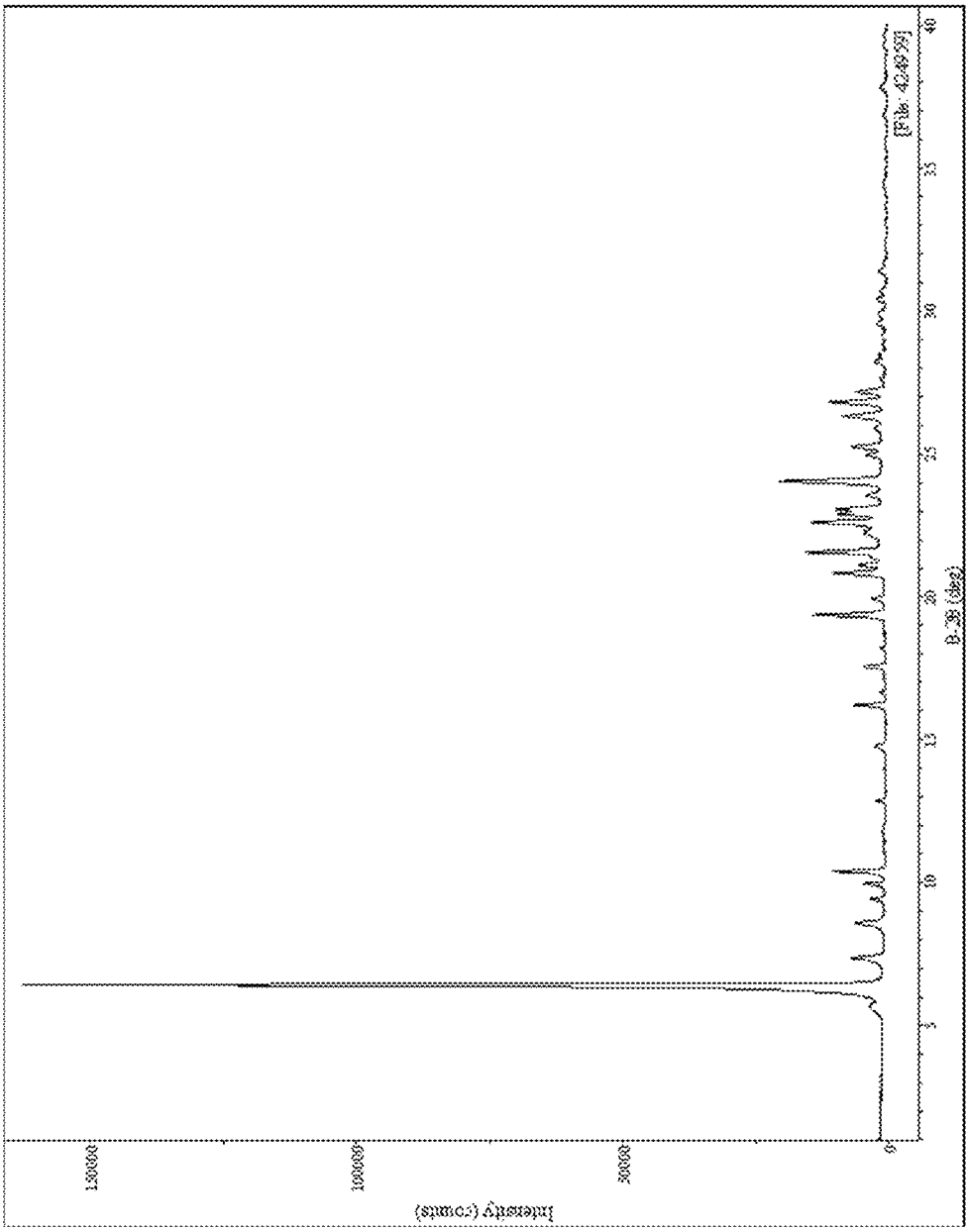

Intensity (counts)
1500000
1000000
500000
0
5
10
15
20
25
30
35
40
2-Theta (deg)
[File: 42.99]

Fig. 31

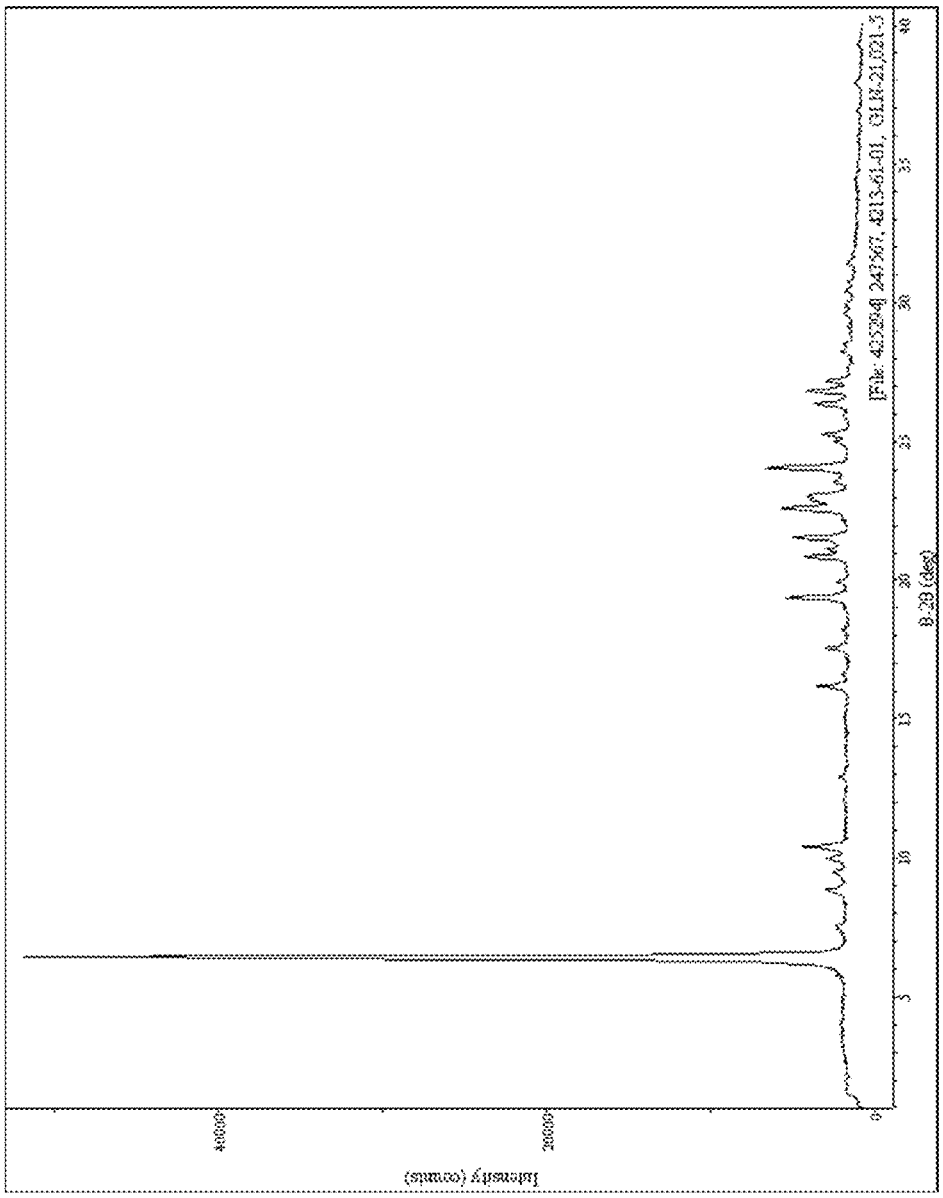

Intensity (counts)
400000
200000
0
5
10
15
20
25
30
35
40
2-Theta (deg)
File: 432294j 247967, 4213-61-01, 01.18-21.021-5

Fig. 32B

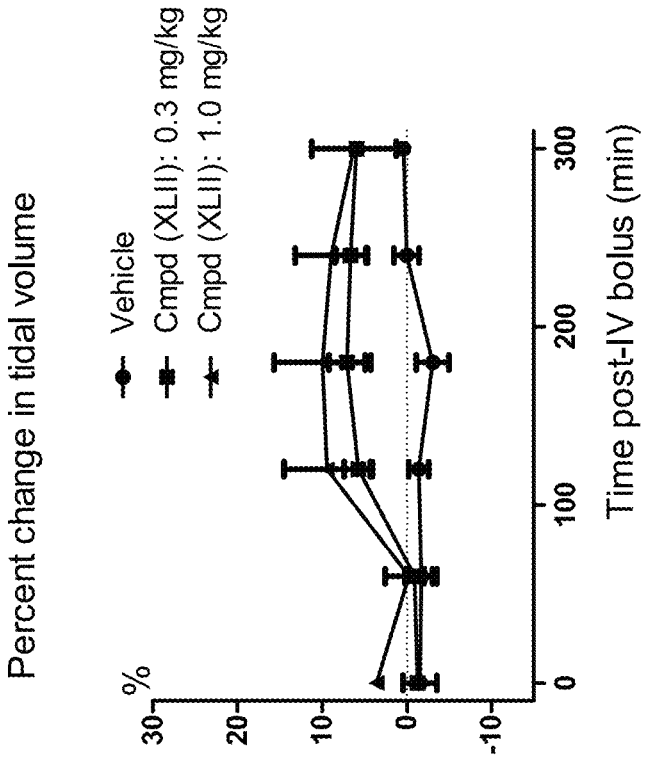


Fig. 32A

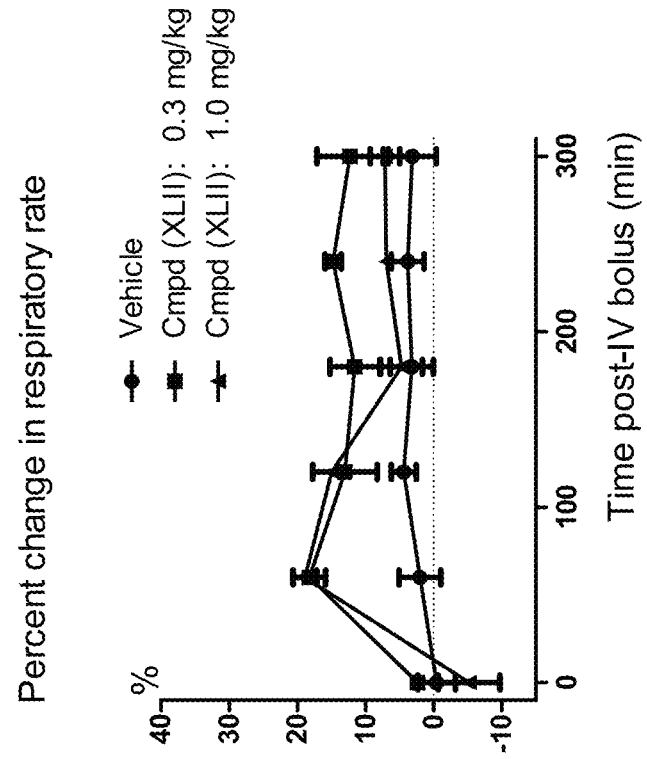


Fig. 32C

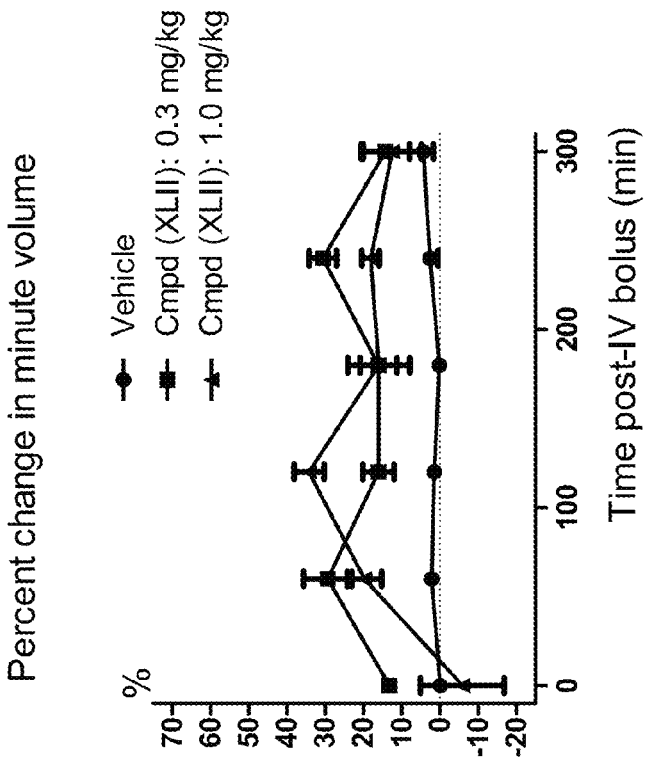


Fig. 32D

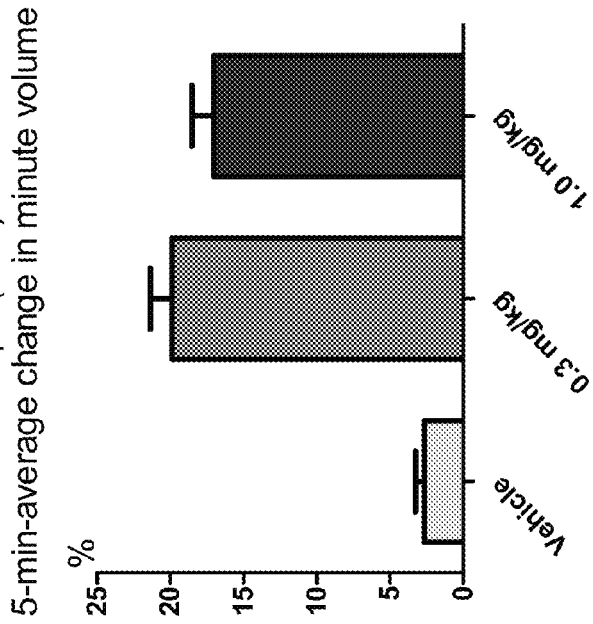


Fig. 33A

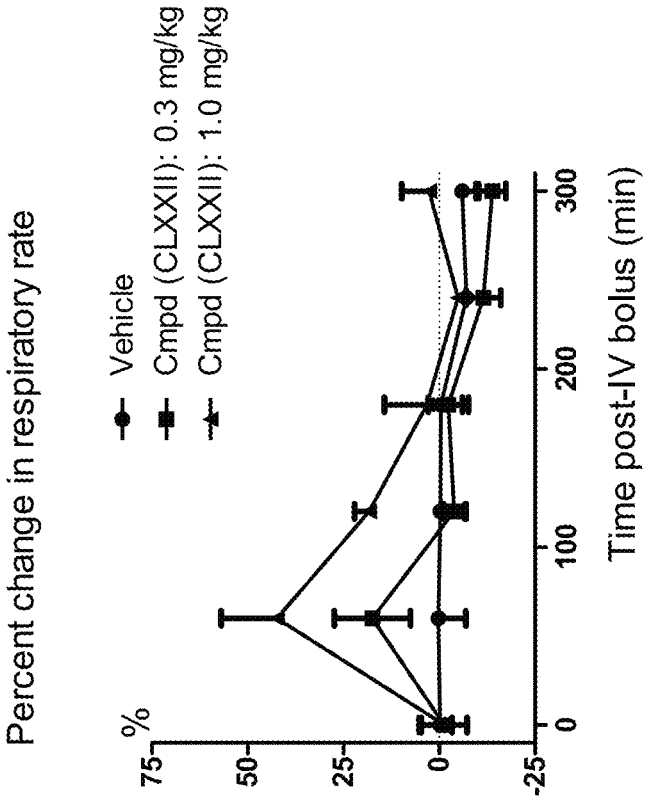


Fig. 33B

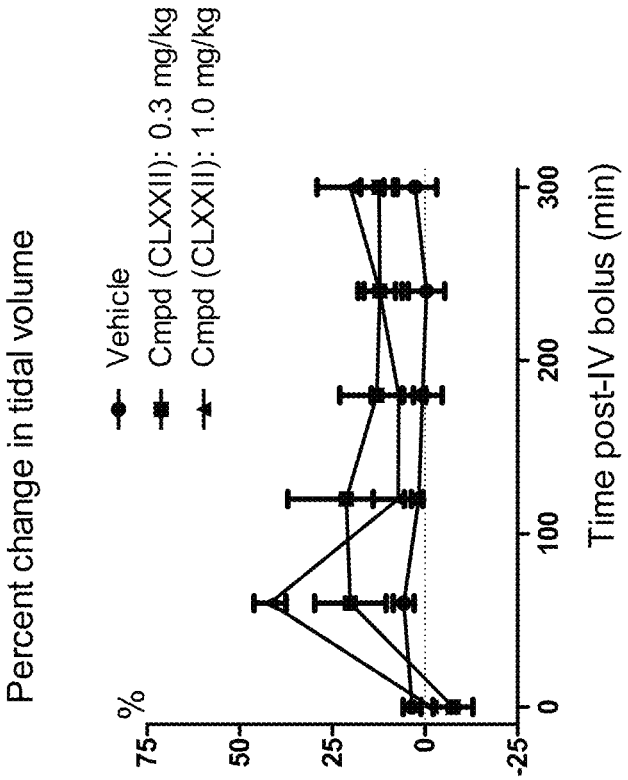


Fig. 33C

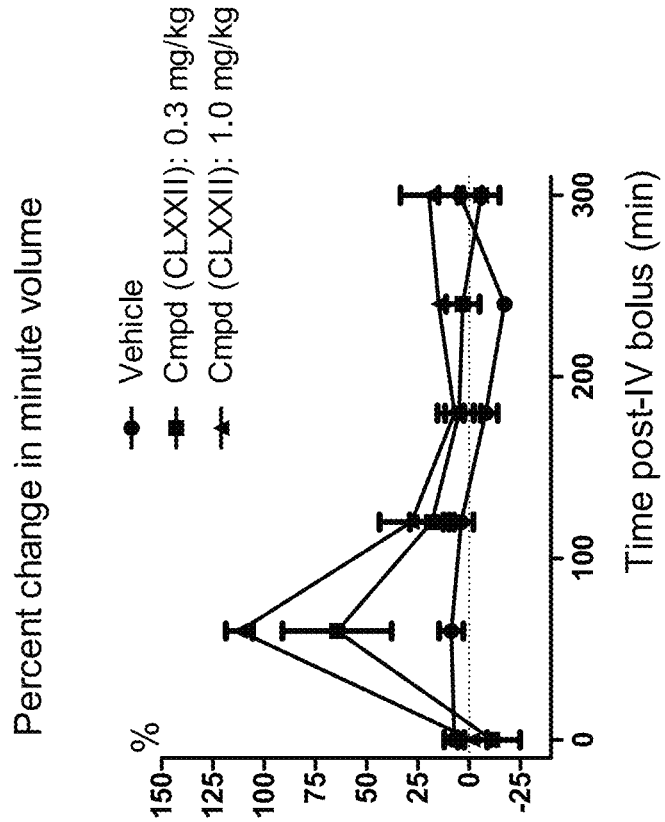


Fig. 33D

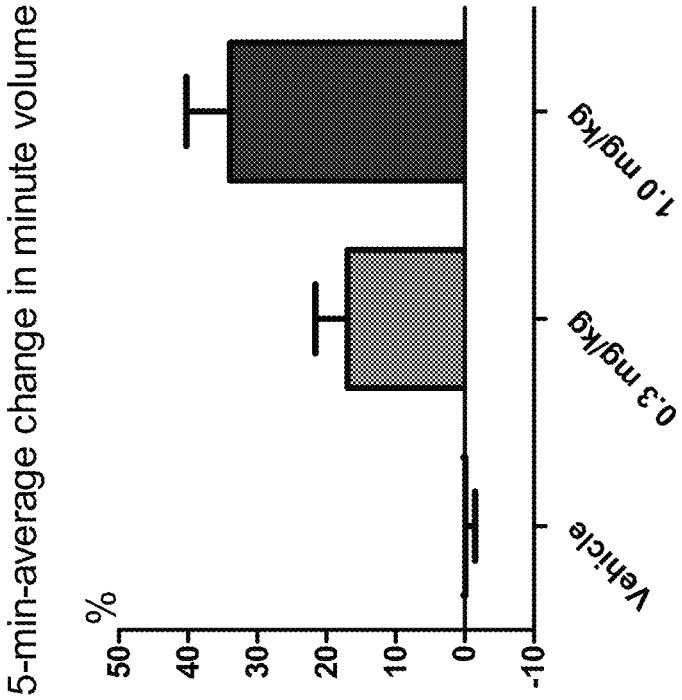


Fig. 34

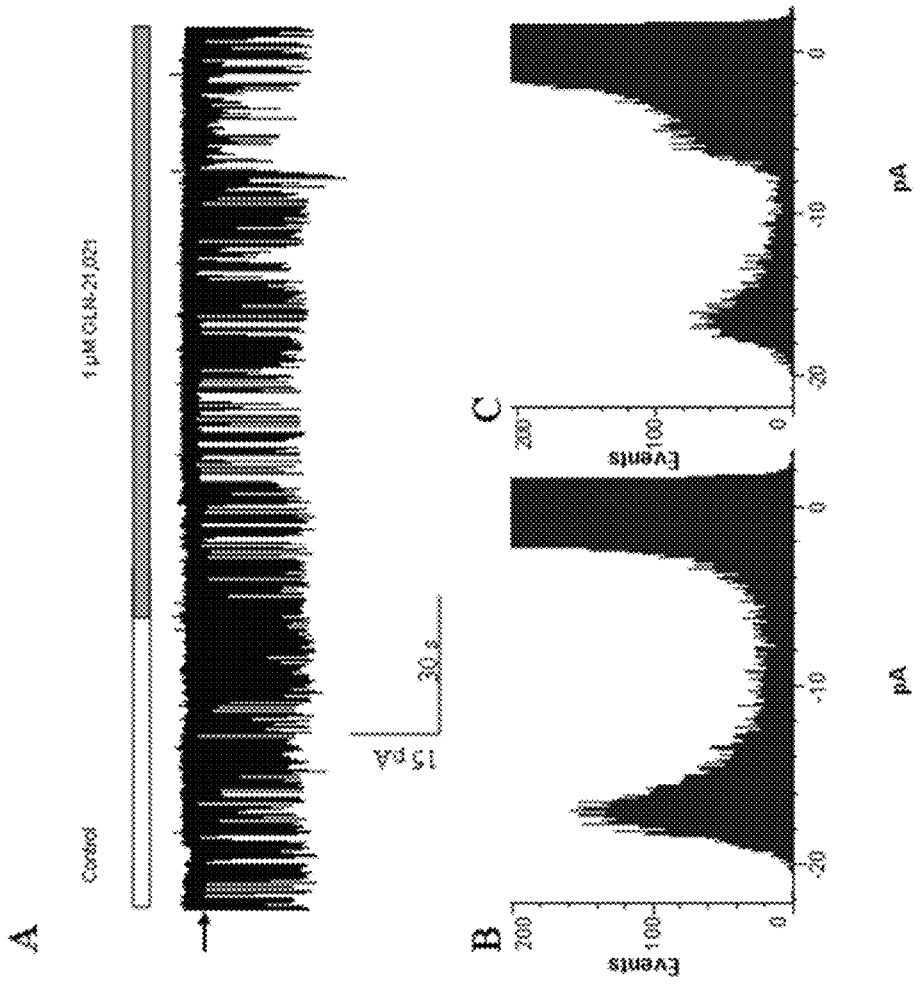
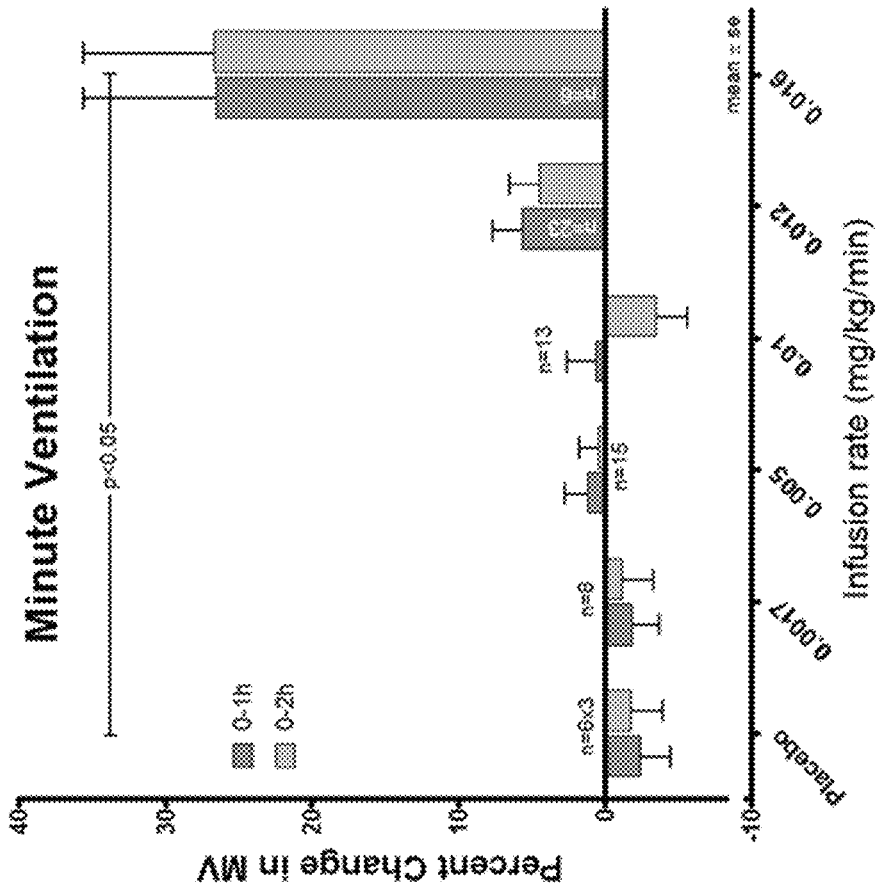

A
Control
1 μM CL218,872
15 pA
30 s
B
Events
200
100
0
-20
-10
0
pA
C
Events
200
100
0
-20
-10
0
pA

Fig. 35


Minute Ventilation
p<0.05
0-1h
0-2h
n=6
n=13
n=15
n=6
n=3
Percent Change in MV
40
30
20
10
0
-10
mean ± se
Placebo
0.0017
0.005
0.01
0.012
0.016
Infusion rate (mg/kg/min)

COMPOUNDS AND COMPOSITIONS FOR TREATMENT OF BREATHING CONTROL DISORDERS OR DISEASES

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application is a continuation of, and claims priority to, U.S. application Ser. No. 13/482,837, filed May 29, 2012, which is a continuation-in-part of, and claims priority to, U.S. patent application Ser. No. 13/306,349, filed Nov. 29, 2011, which is entitled to priority under 35 U.S.C. §119(e) to U.S. Provisional Patent Applications No. 61/417,777, filed Nov. 29, 2010, and No. 61/494,268, filed Jun. 7, 2011, all of which are hereby incorporated by reference in their entireties herein.

BACKGROUND OF THE INVENTION

Normal control of breathing is a complex process that involves, in part, the body's interpretation and response to chemical stimuli such as carbon dioxide, pH and oxygen levels in blood, tissues and the brain. Breathing control is also affected by other factors such as wakefulness (i.e., whether the patient is awake or sleeping), emotion, posture and vocalization. Within the brain medulla, there are respiratory control centers that interpret various feedforward and feedback signals that affect respiration and issues commands to the muscles that perform the work of breathing. Key muscle groups are located in the abdomen, diaphragm, pharynx and thorax. Sensors located centrally and peripherally then provide input to the brain's central respiration control areas that enables response to changing metabolic requirements.

For example, ventilation sufficient to meet the body's metabolic needs is maintained primarily by the body's rapid response to changes in carbon dioxide levels (CO_2). Increased CO_2 levels signal the body to increase breathing rate and depth, resulting in higher blood oxygen levels and subsequent lower blood CO_2 levels. Conversely, low CO_2 levels can result in periods of hyponea (decreased breathing) or, in the extreme case, apnea (no breathing) since the stimulation to breathe is diminished. This is what happens when a person hyperventilates.

There are many diseases in which loss of normal breathing control is a primary or secondary feature of the disease. Examples of diseases with a primary loss of breathing control are apneas (central, mixed or obstructive; where the breathing repeatedly stops for 10 to 60 seconds) and congenital central hypoventilation syndrome. Secondary loss of breathing control may be due to chronic cardio-pulmonary diseases (e.g., heart failure, chronic bronchitis, emphysema, and impending respiratory failure), excessive weight (e.g., obesity-hypoventilation syndrome), certain drugs (e.g., anesthetics, sedatives, anxiolytics, hypnotics, alcohol, and narcotic analgesics and/or factors that affect the neurological system (e.g., stroke, tumor, trauma, radiation damage, and ALS). In chronic obstructive pulmonary diseases where the body is exposed to chronically high levels of carbon dioxide, the body adapts to the lower pH by a kidney mediated retention of bicarbonate, which has the effect of partially neutralizing the CO_2/pH respiratory stimulation. Thus, the patient is unable to mount a normal ventilatory response to changes in metabolic demand.

Sleep disordered breathing is an example of where abnormalities in the control of breathing lead to a serious and prevalent disease in humans. Sleep apnea is characterized by frequent periods of no or partial breathing. Key factors that contribute to these apneas include anatomical factors (such as obesity), decreased hypercapnic and hypoxic ventilatory responses (e.g., decreased response to high carbon dioxide and low oxygen levels, respectively) and loss of "wakefulness" (i.e., drive to pharyngeal dilator muscles). Apneic events result in hypoxia (and the associated oxidative stress) and eventually severe cardiovascular consequences (high blood pressure, stroke, heart attack).

Estimates for U.S. individuals afflicted with conditions wherein there is compromised respiratory control include sleep apneas (15-20 millions); obesity-hypoventilation syndrome (5-10 millions); chronic heart disease (5 millions); chronic obstructive pulmonary disease (COPD)/chronic bronchitis (10 millions); drug-induced hypoventilation (2-5 millions); and mechanical ventilation weaning (0.5 million).

There is a need in the art for novel chemical compounds that can be used to restore all or part of the body's normal breathing control system in response to changes in CO_2 and/or oxygen, with minimal side effects. Such compounds would be of benefit in decreasing the incidence and severity of breathing control disturbances. The present invention addresses and meets these needs.

BRIEF SUMMARY OF THE INVENTION

The invention includes a composition comprising at least one compound selected from the group consisting of: N-(2,4-bis-n-propylamino)-N-(6-methylamino)-[1,3,5]triazine (CLXXII), N-(2,4,6-tris-n-propylamino)-[1,3,5]triazine (CLXXIII), N-(2,4,6-tris-n-propylamino)-1,3-pyrimidine (CLXXIV), N-(2,4-bis-n-propylamino)-N-(6-i-propylamino)-1,3-pyrimidine (CLXXV), a salt thereof, and any combinations thereof.

In one embodiment, the salt is hydrogen sulfate, hydrochloride, phosphate, hydrogen phosphate or dihydrogen phosphate. In another embodiment, the composition further comprises at least one pharmaceutically acceptable carrier.

The invention also includes a method of preventing or treating a breathing control disorder or disease in a subject in need thereof. The method comprises administering to the subject an effective amount of a pharmaceutical formulation comprising at least one pharmaceutically acceptable carrier and at least one compound selected from the group consisting of: N-(2,4-bis-n-propylamino)-N-(6-methylamino)-[1,3,5]triazine (CLXXII), N-(2,4,6-tris-n-propylamino)-[1,3,5]triazine (CLXXIII), N-(2,4,6-tris-n-propylamino)-1,3-pyrimidine (CLXXIV), N-(2,4-bis-n-propylamino)-N-(6-i-propylamino)-1,3-pyrimidine (CLXXV), a salt thereof, and any combinations thereof.

In one embodiment, the breathing control disorder or disease is selected from the group consisting of respiratory depression, sleep apnea, apnea of prematurity, obesity-hypoventilation syndrome, primary alveolar hypoventilation syndrome, dyspnea, hypoxia, and hypercapnia. In another embodiment, the respiratory depression is caused by an anesthetic, a sedative, an anxiolytic agent, a hypnotic agent, alcohol or a narcotic. In yet another embodiment, the subject is further administered a composition comprising at least one additional compound useful for treating the breathing control disorder or disease. In yet another embodiment, the at least one additional compound is selected from the group consisting of acetazolamide, almitrine, theophylline, caffeine, methyl progesterone, a serotinergic modulator, a cannabinoid and an ampakine. In yet another embodiment, the formulation is administered in conjunction with the use of a mechanical ventilation device or positive airway pressure device on the subject. In yet another embodiment, the subject is a mammal. In yet another embodiment, the mammal is a human. In yet

another embodiment, the formulation is administered to the subject by an inhalational, topical, oral, buccal, rectal, vaginal, intramuscular, subcutaneous, transdermal, intrathecal, intraperitoneal, intrathoracic, intrapleural or intravenous route.

The invention also includes a method of preventing destabilization or stabilizing breathing rhythm in a subject in need thereof. The method comprising administering to the subject an effective amount of a pharmaceutical formulation comprising at least one pharmaceutically acceptable carrier and at least one compound selected from the group consisting of: N-(2,4-bis-n-propylamino)-N-(6-methylamino)-[1,3,5]triazine (CLXXII), N-(2,4,6-tris-n-propylamino)-[1,3,5]triazine (CLXXIII), N-(2,4,6-tris-n-propylamino)-1,3-pyrimidine (CLXXIV), N-(2,4-bis-n-propylamino)-N-(6-i-propylamino)-1,3-pyrimidine (CLXXV), a salt thereof, and any combinations thereof.

In one embodiment, the destabilization is associated with a breathing control disorder or disease selected from the group consisting of respiratory depression, sleep apnea, apnea of prematurity, obesity-hypoventilation syndrome, primary alveolar hypoventilation syndrome, dyspnea, hypoxia, and hypercapnia. In another embodiment, the respiratory depression is caused by an anesthetic, a sedative, an anxiolytic agent, a hypnotic agent, alcohol or a narcotic. In yet another embodiment, the subject is further administered a composition comprising at least one additional compound useful for treating the breathing control disorder or disease. In yet another embodiment, the at least one additional compound is selected from the group consisting of acetazolamide, almitrine, theophylline, caffeine, methyl progesterone, a serotinergic modulator, a cannabinoid and an ampakine. In yet another embodiment, the formulation is administered in conjunction with the use of a mechanical ventilation device or positive airway pressure device on the subject. In yet another embodiment, the subject is a mammal. In yet another embodiment, the mammal is a human. In yet another embodiment, the formulation is administered to the subject by an inhalational, topical, oral, buccal, rectal, vaginal, intramuscular, subcutaneous, transdermal, intrathecal, intraperitoneal, intrathoracic, intrapleural or intravenous route.

The invention also includes a composition comprising N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXXV) or a salt thereof, wherein 4,6-bis-(n-propylamino)-1,3,5-triazin-2-ol (CLXXVIII) is present at a level equal to or lower than about 1% (w/w) with respect to (XXXV) or the salt thereof.

In one embodiment, 4,6-bis-(n-propylamino)-1,3,5-triazin-2-ol (CLXXVIII) is present at a level equal to or lower than about 0.5% (w/w) with respect to (XXXV) or the salt thereof. In another embodiment, 4,6-bis-(n-propylamino)-1,3,5-triazin-2-ol (CLXXVIII) is present at a level equal to or lower than about 0.3% (w/w) with respect to (XXXV) or the salt thereof. In yet another embodiment, 4,6-bis-(n-propylamino)-1,3,5-triazin-2-ol (CLXXVIII) is present at a level equal to or lower than about 0.2% (w/w) with respect to (XXXV) or the salt thereof. In yet another embodiment, 4,6-bis-(n-propylamino)-1,3,5-triazin-2-ol (CLXXVIII) is present at a level equal to or lower than about 0.15% (w/w) with respect to (XXXV) or the salt thereof. In yet another embodiment, 4,6-bis-(n-propylamino)-1,3,5-triazin-2-ol (CLXXVIII) is present at a level equal to or lower than about 0.1% (w/w) with respect to (XXXV) or the salt thereof. In yet another embodiment, 4,6-bis-(n-propylamino)-1,3,5-triazin-2-ol (CLXXVIII) is essentially absent from the composition.

The invention also includes a composition comprising N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXXV) or a salt thereof, further comprising a buffer and a liquid carrier.

In one embodiment, the salt of (XXXV) is the hydrogen sulfate salt (XXXVI). In another embodiment, the composition further comprises citric acid. In yet another embodiment, the pH of the composition is adjusted with a base. In yet another embodiment, the base comprises sodium hydroxide. In yet another embodiment, the liquid carrier comprises water. In yet another embodiment, the pH of the composition ranges from about 2.5 to about 6. In yet another embodiment, the pH of the composition ranges from about 2.5 to about 5. In yet another embodiment, the pH of the composition ranges from about 3 to about 4. In yet another embodiment, the concentration of (XXXV) or the salt thereof in the composition is about 1-10 mg/mL. In yet another embodiment, the concentration of (XXXV) or the salt thereof in the composition is about 5-10 mg/mL. In yet another embodiment, the concentration of (XXXV) or the salt thereof in the composition is about 10 mg/mL. In yet another embodiment, the composition comprises less than 0.5% (w/w) (CLXXVIII) with respect to (XXXV). In yet another embodiment, less than 0.5% (w/w) of N-(4,6-Bis-n-propylamino)-[1,3,5]-triazin-2-ol (CLXXVIII) with respect to (XXXV) forms over a six-month period of storage of the composition at 2-8° C. In yet another embodiment, wherein less than 0.5% (w/w) of N-(4,6-Bis-n-propylamino)-[1,3,5]-triazin-2-ol (CLXXVIII) with respect to (XXXV) forms over a twelve-month period of storage of the composition at 2-8° C. In yet another embodiment, less than 0.5% (w/w) of N-(4,6-Bis-n-propylamino)-[1,3,5]-triazin-2-ol (CLXXVIII) with respect to (XXXV) forms over an eighteen-month period of storage of the composition at 2-8° C. In yet another embodiment, less than 0.5% (w/w) of N-(4,6-Bis-n-propylamino)-[1,3,5]-triazin-2-ol (CLXXVIII) with respect to (XXXV) forms over a twenty-four-month period of storage of the composition at 2-8° C.

The invention also includes a crystalline free base of N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXXV), Form A, with a XRPD spectrum as per FIG. **22**.

The invention also includes at least one crystalline salt of N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N.O-dimethyl-hydroxylamine (XXXV) selected from the group consisting of:

(i) crystalline hydrogen sulfate salt (XXXVI) Form A, with a XRPD spectrum as per FIG. **14**;

(ii) crystalline hydrogen sulfate salt (XXXVI) comprising a mixture of Form A and Form B, with a XRPD spectrum as per FIG. **23**;

(iii) crystalline hydrogen sulfate salt (XXXVI) Form C, with a XRPD spectrum as per FIG. **24**;

(iv) crystalline hydrogen sulfate salt (XXXVI) Form D, with a XRPD spectrum as per FIG. **25**;

(v) crystalline hydrogen sulfate salt (XXXVI) comprising a mixture of Form A and Form D, with a XRPD spectrum as per FIG. **26**;

(vi) crystalline phosphoric acid salt (CLXXX) Form A, with a XRPD spectrum as per FIG. **27**;

(vii) crystalline phosphoric acid salt (CLXXX) Form C, with a XRPD spectrum as per FIG. **28**;

(viii) crystalline phosphoric acid salt (CLXXX) comprising a mixture of Form A and Form B, with a XRPD spectrum as per FIG. **29**;

(ix) crystalline phosphoric acid salt (CLXXX) comprising a mixture of Form C and Form D, with a XRPD spectrum as per FIG. **30**;

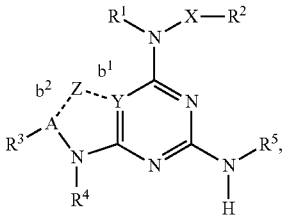
(x) crystalline phosphoric acid salt (CLXXX) comprising a mixture of Form C and Form E, with a XRPD spectrum as per FIG. **31**;

and any mixtures thereof.

The invention also includes a salt of N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-hydroxylamine (XXXV), comprising approximately one mole equivalent of sulfuric acid. In one embodiment, the salt further comprises one mole equivalent of water.

The invention also includes a salt of N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N.O-dimethyl-hydroxylamine (XXXV), comprising at least one mole equivalent of phosphoric acid. In one embodiment, the salt comprises about one mole equivalent of phosphoric acid.

The invention also includes a method of reducing or minimizing the open channel fraction of the potassium maxi-K or BK channel in a subject in need thereof. The method comprises administering to the subject an effective amount of a pharmaceutical formulation comprising at least one pharmaceutically acceptable carrier and at least one compound of formula (I):



(I)

wherein

R^1 and R^2 are independently H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, phenyl, substituted phenyl, phenylalkyl, substituted phenylalkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, heteroarylalkyl, substituted heteroarylalkyl, heteroaryl or substituted heteroaryl; or R^1 and R^2 combine as to form a biradical selected from the group consisting of 3-hydroxy-pentane-1,5-diyl, 6-hydroxy-cycloheptane-1,4-diyl, propane-1,3-diyl, butane-1,4-diyl and pentane-1,5-diyl;

R^3 is H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, —NR^1R^2, —C(O)OR^1, acyl, or aryl;

R^4 is H, alkyl, or substituted alkyl;

R^5 is H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, —OR^1, —NR^1R^2, —C(O)OR^1, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, or substituted heterocyclic; or R^3 and R^5 combine as to form a biradical selected from the group consisting of 3,6,9-trioxa-undecane-1,11-diyl and 3,6-dioxa-octane-1,8-diyl;

R^6 is H, alkyl, substituted alkyl or alkenyl;

X is a bond, O or NR^4; and,

Y is N, CR^6 or C; wherein:

if Y is N or CR^6, then bond b^1 is nil and:

(i) Z is H, bond b^2 is a single bond, and A is CH; or,

(ii) Z is nil, bond b^2 is nil, and A is a single bond;

and,

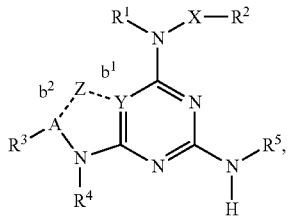
if Y is C, then bond b^1 is a single bond, and:

(i) Z is CH_2, bond b^2 is a single bond, and A is CH; or,

(ii) Z is CH, bond b^2 is a double bond, and A is C;

or a salt thereof.

The invention also includes a method of inhibiting the TASK-1 (KCNK3) channel in a subject in need thereof. The method comprises administering to the subject an effective amount of a pharmaceutical formulation comprising at least one pharmaceutically acceptable carrier and at least one compound of formula (I):



(I)

wherein

R^1 and R^2 are independently H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, phenyl, substituted phenyl, phenylalkyl, substituted phenylalkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, heteroarylalkyl, substituted heteroarylalkyl, heteroaryl or substituted heteroaryl; or R^1 and R^2 combine as to form a biradical selected from the group consisting of 3-hydroxy-pentane-1,5-diyl, 6-hydroxy-cycloheptane-1,4-diyl, propane-1,3-diyl, butane-1,4-diyl and pentane-1,5-diyl;

R^3 is H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, —NR^1R^2, —C(O)OR^1, acyl, or aryl;

R^4 is H, alkyl, or substituted alkyl;

R^5 is H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, —OR^1, —NR^1R^2, —C(O)OR^1, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, or substituted heterocyclic; or R^3 and R^5 combine as to form a biradical selected from the group consisting of 3,6,9-trioxa-undecane-1,11-diyl and 3,6-dioxa-octane-1,8-diyl;

R^6 is H, alkyl, substituted alkyl or alkenyl;

X is a bond, O or NR^4; and,

Y is N, CR^6 or C; wherein:

if Y is N or CR^6, then bond b^1 is nil and:

(i) Z is H, bond b^2 is a single bond, and A is CH; or,

(ii) Z is nil, bond b^2 is nil, and A is a single bond;

and,

if Y is C, then bond b^1 is a single bond, and:

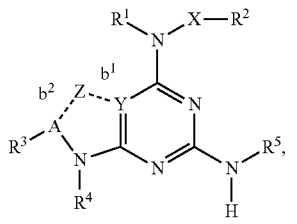
(i) Z is CH_2, bond b^2 is a single bond, and A is CH; or,

(ii) Z is CH, bond b^2 is a double bond, and A is C;

or a salt thereof.

The invention also includes a method of increasing minute ventilation in a subject in need thereof. The method comprises administering to the subject an effective amount of a pharmaceutical formulation comprising at least one pharmaceutically acceptable carrier and at least one compound of formula (I):

(I)



wherein

R^1 and R^2 are independently H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, phenyl, substituted phenyl, phenylalkyl, substituted phenylalkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, heteroarylalkyl, substituted heteroarylalkyl, heteroaryl or substituted heteroaryl; or R^1 and R^2 combine as to form a biradical selected from the group consisting of 3-hydroxy-pentane-1,5-diyl, 6-hydroxy-cycloheptane-1,4-diyl, propane-1,3-diyl, butane-1,4-diyl and pentane-1,5-diyl;

R^3 is H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, —NR^1R^2, —$C(O)OR^1$, acyl, or aryl;

R^4 is H, alkyl, or substituted alkyl;

R^5 is H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, —OR^1, —NR^1R^2, —$C(O)OR^1$, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, or substituted heterocyclic; or R^3 and R^5 combine as to form a biradical selected from the group consisting of 3,6,9-trioxa-undecane-1,11-diyl and 3,6-dioxa-octane-1,8-diyl;

R^6 is H, alkyl, substituted alkyl or alkenyl;

X is a bond, O or NR^4; and,

Y is N, CR^6 or C; wherein:

if Y is N or CR^6, then bond b^1 is nil and:

(i) Z is H, bond b^2 is a single bond, and A is CH; or,

(ii) Z is nil, bond b^2 is nil, and A is a single bond;

and,

if Y is C, then bond b^1 is a single bond, and:

(i) Z is CH_2, bond b^2 is a single bond, and A is CH; or,

(ii) Z is CH, bond b^2 is a double bond, and A is C;

or a salt thereof.

In one embodiment, the compound of formula (I) is selected from the group consisting of: N-(4,6-Bis-methylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(4,6-Bis-ethylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(4-Cyclopropylmethyl)-N-(6-n-propylamino)[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(4-Ethylamino)-N-(6-n-propylamino)-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(Bis-4,6-(2-methylpropylamino)) [1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(Bis-4,6-(2,2-dimethylpropylamino)) [1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(Bis-4,6-(2,2-dimethylpropylamino)) [1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(4-(Methoxy(methyl)amino)-6-(n-propylamino)-1,3,5-triazin-2-yl) propionamide, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-methyl-hydroxylamine, O-Allyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-hydroxylamine, 6-(Methoxy(methyl)amino)-N^2-propyl-1,3,5-triazine-2,4-diamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N-methyl-hydroxylamine, O-Benzyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N-methyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N-isopropyl-hydroxylamine, 6-[1,2]Oxazinan-2-yl-N,N'-dipropyl-[1,3,5]triazine-2,4-diamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isopropyl-N-methyl-hydroxylamine, O-Benzyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N-ethyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isopropyl-hydroxylamine, 6-((Benzyloxy)(isopropyl)amino)-N^2,N^4-dipropyl-1,3,5-triazine-2,4-diamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N-ethyl-O-isopropyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isobutyl-N-methyl-hydroxylamine, 6-(Methyl(thiophen-2-ylmethoxy)amino)-N^2,N^4-dipropyl-1,3,5-triazine-2,4-diamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-cyclopropylmethyl-N-methyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-ethyl-N-methyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-(2,2-difluoro-ethyl)-hydroxylamine, 4-N-(2-Dimethylaminoethyl)amino-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, 4-N-(3-(1-N-Methylimidazol-2-yl)-propyl)-amino-6-N-(n-propyl) amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, 4-N-(1-N-Methylimidazol-2-yl)-methylamino-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, 4,6-Bis-(N-(2-dimethylaminoethyl)amino)-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, 4,6-Bis-(N-(pyridin-4-yl-methyl)amino)-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, 4,6-Bis-[N-(3-methoxy-n-propyl) amino]-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, 4,6-Bis-[N-(tetrahydropyran-4-yl-methyl)amino]-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(5,8,11-Trioxa-2,14,16,18,19-pentaazabicyclo[13.3.1]nonadeca-1(18),15(19),16(17)-trien-17-yl)-N,O-dimethylhydroxylamine, 2,6-Bis-(N-n-propylamino)-[1,3]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N',N'-dimethylhydrazine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N-methyl-N'-methylhydrazine, 2-(n-Propyl)amino-4-(i-propylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine, 2-(n-Propyl)amino-4-dimethylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine, 2-(n-Propyl)amino-4-methylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine, 2-(n-Propyl)amino-4-(i-propyl)amino-7-i-propyl-pyrrolidino[2,3-d]pyrimidine, 2,4-Bis-(n-propyl) amino-7H-pyrrolidino[2,3-d]pyrimidine, 2-(n-Propyl) amino-4-(4-hydroxypiperidin-1-yl)-7-methyl-pyrrolidino[2,3-d]pyrimidine, 8-(7-Methyl-2-(n-propylamino)-pyrrolidino [2,3-d]pyrimidin-4-yl)-8-azabicyclo[3.2.1]octan-3-ol, N-(2-Propylamino-7H-pyrrolo[2,3d]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine, N-(2-(Propen-2-yl)amino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine, N-(2-(Propen-2-yl)amino-7-methyl-pyrrolo [2,3d]pyrimidin-4-yl)-O-methyl-hydroxylamine, N-(2-n-Propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine, N-(2-n-Propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-O-methyl-hydroxylamine, N-(2-n-Propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine, N-Methyl-N-(2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine, N,N-Dimethyl-N'-(2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine, N-(2,4-Bis-n-propylamino)-N-(6-methylamino)-[1,3,5]triazine, N-(2,4,6-Tris-n-propylamino)-[1,3,5]triazine, N-(2,4,6-Tris-n-propylamino)-1,3-pyrimidine, N-(2,4-Bis-n-propylamino)-N-(6-i-propylamino)-1,3-pyrimidine, a salt thereof and mixtures thereof.

In one embodiment, the compound of formula (I) is selected from the group consisting of: N-(4,6-Bis-n-propylamino-1,3,5]triazin-2-yl)-O-methyl-hydroxylamine; N-(4,6-Bis-n-propylamino-1,3,5]triazin- 2-yl)-N',N'-dimethylhy-

drazine; N-(2,4-Bis-n-propylamino)-N-(6-methylamino)-[1,3,5]triazine; N-[(2,6-Bis-n-propylamino)-pyrimidin-4-yl]-N,O-dimethyl-hydroxylamine; N,N-Dimethyl-N'-(2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine; a salt thereof and mixtures thereof.

In one embodiment, the compound of formula (I) is N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, or a salt thereof.

In one embodiment, the pharmaceutical formulation is administered via intravenous infusion. In another embodiment, the infusion dose of the pharmaceutical formulation is at least about 0.016 mg/kg/min. In yet another embodiment, the infusion dose of the pharmaceutical formulation is about 0.016 mg/kg/min. In yet another embodiment, the infusion dose of the pharmaceutical composition produce plasma concentrations of at least about 726 ng/mL in the subject. In yet another embodiment, the subject is a mammal. In yet another embodiment, the mammal is human.

BRIEF DESCRIPTION OF THE DRAWINGS

For the purpose of illustrating the invention, there are depicted in the drawings certain embodiments of the invention. However, the invention is not limited to the precise arrangements and instrumentalities of the embodiments depicted in the drawings.

FIG. **1** is a graph illustrating results of plethysmography experiments which monitored minute ventilation in the opioid-treated rat upon administration of Compound (XXXVI) [labeled as cmpd (A)].

FIG. **2**, comprising FIGS. **2**A-**2**B, illustrates arterial blood gas analysis results for administration of Compound (XXXVI) [labeled as cmpd (A)] in the opioid-treated rat. FIG. **2**A—$PaCO_2$ (mmHg); FIG. **2**B—SaO_2 (%).

FIG. **3** is a graph illustrating results of plethysmography experiments monitoring minute ventilation in the rat upon administration of Compound (XXXVI) [labeled as cmpd (A)] under conditions of hypoxia.

FIG. **4** is a graph illustrating end-tidal CO_2 and minute ventilation in the opioid-treated monkey upon administration of Compound (XXXVI) [labeled as cmpd (A)].

FIG. **5** is a graph illustrating the dose-dependent effect of Compound (XXXVI) [labeled as cmpd (A)] and Compound (L) [labeled as cmpd (B)] on minute ventilation, in terms of maximum peak response, in the rat.

FIG. **6** is a graph illustrating the dose-dependent effect of Compound (XXXVI) [labeled as cmpd (A)] and Compound (CXXI) [labeled as cmpd (C)] on minute ventilation, in terms of maximum peak response, in the rat.

FIG. **7**, comprising FIGS. **7**A-**7**D, illustrates the dose-dependent effect of Compound (L) [labeled as cmpd (B)] on blood gases and pH in the opioid-treated rat. FIG. **7**A—pH; FIG. **7**B—SaO_2; FIG. **7**C—pO_2; FIG. **7**D—pCO_2.

FIG. **8**, comprising FIGS. **8**A-**8**D, illustrates the dose-dependent effect of Compound (CXLII) [labeled as cmpd (D)] on blood gases and pH in the opioid-treated rat. FIG. **8**A—pO_2; FIG. **8**B—SaO_2; FIG. **8**C—pCO_2; FIG. **8**D—pH.

FIG. **9** is a graph illustrating the effect of Compound (CXLII) [labeled as cmpd (D)] in the minute ventilation of the opioid-treated rat.

FIG. **10** illustrates the ^{1}H-NMR spectrum for Compound (XXXVI) in DMSO-d_6 at 25° C.

FIG. **11** illustrates the ^{13}C-NMR spectrum for Compound (XXXVI) in DMSO-d_6 at 25° C.

FIG. **12** illustrates the FTIR spectrum for Compound (XXXVI).

FIG. **13** illustrates the thermal analysis by DSC of N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrogen sulfate (XXXVI).

FIG. **14** illustrates the X-ray diffraction spectrum of Compound (XXXVI) Form A (hydrogen sulfate salt Form A).

FIG. **15** is a graph illustrating the effect of Compound (XXXVI) on reversing the effects of midazolam on minute ventilation (MV) in the rat.

FIG. **16** is a graph illustrating the effect of Compound (XXXVI) on reversing the effects of midazolam on tidal volume (TV) in the rat.

FIG. **17** is a graph illustrating the effect of Compound (XXXVI) on reversing the effects of midazolam on respiratory frequency (f) in the rat.

FIG. **18** is a graph illustrating the effect of Compound (XXXVI) infusion on the minute volume response to acute hypercapnia (3% CO_2).

FIG. **19**A is a graph illustrating the effect of pH on aqueous solubility of Compound (XXXV) at ambient temperature. FIG. **19**B is a graph illustrating the effect of pH on aqueous solubility of Compound (XXXV) at ambient temperature

FIG. **20**A is a graph illustrating the effect of pH on degradation of Compound (XXXV) at pH 2-6. FIG. **20**B is a graph illustrating the effect of pH on degradation of Compound (XXXV) at pH 2-3.6.

FIG. **21** is a graph illustrating the formation rate of Compound (CLXXVIII) from Compound (XXXV) in solution at 2-8° C., pH 3-4.

FIG. **22** is a graph illustrating the X-ray powder diffraction pattern of the free base of Compound (XXXV)

FIG. **23** is a graph illustrating the X-ray powder diffraction spectrum of a mixture of Compound (XXXVI) Form A and Compound (XXXVI) Form B.

FIG. **24** is a graph illustrating the X-ray powder diffraction spectrum of Compound (XXXVI) Form C.

FIG. **25** is a graph illustrating the X-ray powder diffraction spectrum of Compound (XXXVI) Form D.

FIG. **26** is a graph illustrating the X-ray powder diffraction spectrum of a mixture of Compound (XXXVI) Form A and Compound (XXXVI) Form D.

FIG. **27** is a graph illustrating the X-ray powder diffraction spectrum of Compound (CLXXX) Form A.

FIG. **28** is a graph illustrating the X-ray powder diffraction spectrum of Compound (CLXXX) Form C.

FIG. **29** is a graph illustrating the X-ray powder diffraction spectrum of a mixture of Compound (CLXXX) Form A and Compound (CLXXX) Form B.

FIG. **30** is a graph illustrating the X-ray powder diffraction spectrum of a mixture of Compound (CLXXX) Form C and Compound (CLXXX) Form D.

FIG. **31** is a graph illustrating the X-ray powder diffraction spectrum of a mixture of Compound (CLXXX) Form C and Compound (CLXXX) Form E.

FIG. **32**, comprising FIGS. **32**A-**32**D, are graphs illustrating the effect of Compound (XLII) on respiratory rate (FIG. **32**A), tidal volume (FIG. **32**B), minute volume (FIG. **32**C) and 5 min average change in minute volume (FIG. **32**D) respectively, in rat.

FIG. **33**, comprising FIGS. **33**A-**33**D, are graphs illustrating the effect of Compound (CLXXII) on respiratory rate (FIG. **33**A), tidal volume (FIG. **33**B), minute volume (FIG. **33**C) and 5 min average change in minute volume (FIG. **33**D) respectively, in rat.

FIG. **34**, comprising FIGS. **34**A-**34**C, illustrates the effect of BK channel activity in response to Compound (XXXV) at a concentration of 1 μM. FIG. **34**A: sample recordings of channel activity in control conditions and with 1 μM

(XXXV). Arrow, closed state. FIG. **34**B: all points histogram obtained from 1 min of recording in control condition. FIG. **34**C: all points histogram obtained from 1 min of recording with 1 μM (XXXV). Patch ID: C 05 SP 101008_0000.

FIG. **35** is a graph illustrating the effects of Cmpd (XXXVI) on the minute ventilation in human volunteers based upon dosage.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates in one aspect to the unexpected discovery that the compounds of the invention are respiratory stimulants and useful in the treatment of breathing control disorders or diseases.

DEFINITIONS

As used herein, each of the following terms has the meaning associated with it in this section.

Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Generally, the nomenclature used herein and the laboratory procedures in animal pharmacology, pharmaceutical science, separation science and organic chemistry are those well-known and commonly employed in the art.

As used herein, the articles "a" and "an" refer to one or to more than one (i.e. to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

As used herein, the term "about" is understood by persons of ordinary skill in the art and varies to some extent on the context in which it is used. As used herein when referring to a measurable value such as an amount, a temporal duration, and the like, the term "about" is meant to encompass variations of ±20% or ±10%, more preferably ±5%, even more preferably ±1%, and still more preferably ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

As used herein, a "subject" may be a human or non-human mammal. Non-human mammals include, for example, livestock and pets, such as ovine, bovine, porcine, canine, feline and murine mammals. Preferably, the subject is human.

In a non-limiting embodiment, the following terminology used to report blood gas measurements is well known to those skilled in the art and may be defined as such: minute ventilation (MV) is a measure of breathing volume per unit time and is given herein as mL/min; pCO_2 is partial pressure of carbon dioxide (gas) in (arterial) blood measured in mm Hg (millimeters of Hg); pO_2 is partial pressure of oxygen (gas) in (arterial) blood measured in mmHg (millimeters of Hg); SaO_2 is the percentage of hemoglobin in the form of oxyhemoglobin in blood; end-tidal CO_2 is the measurement of exhaled carbon dioxide gas as detected using colorimetry, or capnometry.

As used herein, the term ED_{50} refers to the effective dose of a formulation that produces a given effect in 50% of the subjects that are administered that formulation.

As used herein, a "disease" is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal's health continues to deteriorate.

As used herein, a "disorder" in an animal is a state of health in which the animal is able to maintain homeostasis, but in which the animal's state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal's state of health.

As used herein, an "effective amount", "therapeutically effective amount" or "pharmaceutically effective amount" of a compound is that amount of compound that is sufficient to provide a beneficial effect to the subject to which the compound is administered. The term to "treat," as used herein, means reducing the frequency with which symptoms are experienced by a patient or subject or administering an agent or compound to reduce the severity with which symptoms are experienced.

As used herein, the term "pharmaceutically acceptable" refers to a material, such as a carrier or diluent, which does not abrogate the biological activity or properties of the compound useful within the invention, and is relatively non-toxic, i.e., the material may be administered to an individual without causing undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

As used herein, the term "salt" refers to an addition salt of free acid or base that is useful within the methods of the invention. The term "salt" also refers to a mixture of a salt with its corresponding free acid or base, either in isolated form (e.g., as a solid) or in solution.

As used herein, the language "pharmaceutically acceptable salt" refers to a salt of the administered compound prepared from pharmaceutically acceptable non-toxic acids and bases, including inorganic acids, inorganic bases, organic acids, inorganic bases, solvates, hydrates, and clathrates thereof.

As used herein, the term "composition" or "pharmaceutical composition" refers to a mixture of at least one compound useful within the invention with a pharmaceutically acceptable carrier. The pharmaceutical composition facilitates administration of the compound to a subject.

As used herein, the term "pharmaceutically acceptable carrier" means a pharmaceutically acceptable material, composition or carrier, such as a liquid or solid filler, stabilizer, dispersing agent, suspending agent, diluent, excipient, thickening agent, solvent or encapsulating material, involved in carrying or transporting a compound useful within the invention within or to the subject such that it may perform its intended function. Typically, such constructs are carried or transported from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation, including the compound useful within the invention, and not injurious to the subject. Some examples of materials that may serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; surface active agents; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations. As used herein, "pharmaceutically acceptable carrier" also includes any and all coatings, antibacterial and antifungal agents, and absorption delaying agents, and the like that are compatible with the

activity of the compound useful within the invention, and are physiologically acceptable to the subject. Supplementary active compounds may also be incorporated into the compositions. The "pharmaceutically acceptable carrier" may further include a pharmaceutically acceptable salt of the compound useful within the invention. Other additional ingredients that may be included in the pharmaceutical compositions used in the practice of the invention are known in the art and described, for example in Remington's Pharmaceutical Sciences (Genaro, Ed., Mack Publishing Co., 1985, Easton, Pa.), which is incorporated herein by reference.

As used herein, the phrase "process-related impurities" refers to impurities formed during the process of making the active pharmaceutical ingredient, as caused by exposure of any starting material, intermediate, reactant, solvent, and the active pharmaceutical ingredient itself to the conditions of the synthetic process.

As used herein, "treating a disease or disorder" means reducing the frequency with which a symptom of the disease or disorder is experienced by a subject. Disease and disorder are used interchangeably herein.

By the term "specifically bind" or "specifically binds," as used herein, is meant that a first molecule preferentially binds to a second molecule (e.g., a particular receptor or enzyme), but does not necessarily bind only to that second molecule.

As used herein, the term "alkyl," by itself or as part of another substituent means, unless otherwise stated, a straight or branched chain hydrocarbon having the number of carbon atoms designated (i.e. C_1-C_{10} means one to ten carbon atoms) and includes straight, branched chain, or cyclic substituent groups. Examples include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, neopentyl, hexyl, and cyclopropylmethyl. Most preferred is (C_1-C_6)alkyl, such as, but not limited to, ethyl, methyl, isopropyl, isobutyl, n-pentyl, n-hexyl and cyclopropylmethyl.

As used herein, the term "cycloalkyl," by itself or as part of another substituent means, unless otherwise stated, a cyclic chain hydrocarbon having the number of carbon atoms designated (i.e. C_3-C_6 means a cyclic group comprising a ring group consisting of three to six carbon atoms) and includes straight, branched chain or cyclic substituent groups. Examples include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. Most preferred is (C_3-C_6)cycloalkyl, such as, but not limited to, cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

As used herein, the term "alkenyl," employed alone or in combination with other terms, means, unless otherwise stated, a stable mono-unsaturated or di-unsaturated straight chain or branched chain hydrocarbon group having the stated number of carbon atoms. Examples include vinyl, propenyl (or allyl), crotyl, isopentenyl, butadienyl, 1,3-pentadienyl, 1,4-pentadienyl, and the higher homologs and isomers. A functional group representing an alkene is exemplified by —CH_2—CH=CH_2.

As used herein, the term "alkynyl," employed alone or in combination with other terms, means, unless otherwise stated, a stable straight chain or branched chain hydrocarbon group with a triple carbon-carbon bond, having the stated number of carbon atoms. Examples include ethynyl and propynyl, and the higher homologs and isomers.

As used herein, the term "substituted alkyl," "substituted cycloalkyl," "substituted alkenyl" or "substituted alkynyl" means alkyl, cycloalkyl, alkenyl or alkynyl, as defined above, substituted by one, two or three substituents selected from the group consisting of halogen, —OH, alkoxy, tetrahydro-2-H-pyranyl, —NH_2, —$N(CH_3)_2$, (1-methyl-imidazol-2-yl), pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, —C(=O)OH, trifluoromethyl, —C≡N, —C(=O)O(C_1-C_4)alkyl, —C(=O)NH_2, —C(=O)NH(C_1-C_4)alkyl, —C(=O)N((C_1-C_4)alkyl)$_2$, —SO_2NH_2, —C(=NH)NH_2, and —NO_2, preferably containing one or two substituents selected from halogen, —OH, alkoxy, —NH_2, trifluoromethyl, —$N(CH_3)_2$, and —C(=O)OH, more preferably selected from halogen, alkoxy and —OH. Examples of substituted alkyls include, but are not limited to, 2,2-difluoropropyl, 2-carboxycyclopentyl and 3-chloropropyl.

As used herein, the term "alkoxy" employed alone or in combination with other terms means, unless otherwise stated, an alkyl group having the designated number of carbon atoms, as defined above, connected to the rest of the molecule via an oxygen atom, such as, for example, methoxy, ethoxy, 1-propoxy, 2-propoxy (isopropoxy) and the higher homologs and isomers. Preferred are (C_1-C_3)alkoxy, such as, but not limited to, ethoxy and methoxy.

As used herein, the term "halo" or "halogen" alone or as part of another substituent means, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom, preferably, fluorine, chlorine, or bromine, more preferably, fluorine or chlorine.

As used herein, the term "heteroalkyl" by itself or in combination with another term means, unless otherwise stated, a stable straight or branched chain alkyl group consisting of the stated number of carbon atoms and one or two heteroatoms selected from the group consisting of O, N, and S, and wherein the nitrogen and sulfur atoms may be optionally oxidized and the nitrogen heteroatom may be optionally quaternized. The heteroatom(s) may be placed at any position of the heteroalkyl group, including between the rest of the heteroalkyl group and the fragment to which it is attached, as well as attached to the most distal carbon atom in the heteroalkyl group. Examples include: —O—CH_2—CH_2—CH_3, —CH_2—CH_2—CH_2—OH, —CH_2—CH_2—NH—CH_3, —CH_2—S—CH_2—CH_3, and —CH_2CH_2—S(=O)—CH_3. Up to two heteroatoms may be consecutive, such as, for example, —CH_2—NH—OCH_3, or —CH_2—CH_2—S—S—CH_3

As used herein, the term "heteroalkenyl" by itself or in combination with another term means, unless otherwise stated, a stable straight or branched chain monounsaturated or di-unsaturated hydrocarbon group consisting of the stated number of carbon atoms and one or two heteroatoms selected from the group consisting of O, N, and S, and wherein the nitrogen and sulfur atoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. Up to two heteroatoms may be placed consecutively. Examples include —CH=CH—O—CH_3, —CH=CH—CH_2—OH, —CH_2—CH=N—OCH_3, —CH=CH—$N(CH_3)$—CH_3, and —CH_2—CH=CH—CH_2—SH.

As used herein, the term "aromatic" refers to a carbocycle or heterocycle with one or more polyunsaturated rings and having aromatic character, i.e. having (4n+2) delocalized π (pi) electrons, where n is an integer.

As used herein, the term "aryl," employed alone or in combination with other terms, means, unless otherwise stated, a carbocyclic aromatic system containing one or more rings (typically one, two or three rings) wherein such rings may be attached together in a pendent manner, such as a biphenyl, or may be fused, such as naphthalene. Examples include phenyl, anthracyl, and naphthyl. Preferred are phenyl and naphthyl, most preferred is phenyl.

As used herein, the term "aryl-(C_1-C_3)alkyl" means a functional group wherein a one to three carbon alkylene chain is attached to an aryl group, e.g., —CH_2CH_2-phenyl or —CH_2-phenyl (benzyl). Preferred is aryl-CH_2— and aryl-CH

(CH_3)—. The term "substituted aryl-(C_1-C_3)alkyl" means an aryl-(C_1-C_3)alkyl functional group in which the aryl group is substituted. Preferred is substituted aryl(CH_2)—. Similarly, the term "heteroaryl-(C_1-C_3)alkyl" means a functional group wherein a one to three carbon alkylene chain is attached to a heteroaryl group, e.g., —CH_2CH_2-pyridyl. Preferred is heteroaryl-(CH_2)—. The term "substituted heteroaryl-(C_1-C_3) alkyl" means a heteroaryl-(C_1-C_3)alkyl functional group in which the heteroaryl group is substituted. Preferred is substituted heteroaryl-(CH_2)—.

As used herein, the term "heterocycle" or "heterocyclyl" or "heterocyclic" by itself or as part of another substituent means, unless otherwise stated, an unsubstituted or substituted, stable, mono- or multi-cyclic heterocyclic ring system that consists of carbon atoms and at least one heteroatom selected from the group consisting of N, O, and S, and wherein the nitrogen and sulfur heteroatoms may be optionally oxidized, and the nitrogen atom may be optionally quaternized. The heterocyclic system may be attached, unless otherwise stated, at any heteroatom or carbon atom that affords a stable structure. A heterocycle may be aromatic or non-aromatic in nature. In one embodiment, the heterocycle is a heteroaryl.

As used herein, the term "heteroaryl" or "heteroaromatic" refers to a heterocycle having aromatic character. A polycyclic heteroaryl may include one or more rings that are partially saturated. Examples include tetrahydroquinoline and 2,3-dihydrobenzofuryl.

Examples of non-aromatic heterocycles include monocyclic groups such as aziridine, oxirane, thiirane, azetidine, oxetane, thietane, pyrrolidine, pyrroline, imidazoline, pyrazolidine, dioxolane, sulfolane, 2,3-dihydrofuran, 2,5-dihydrofuran, tetrahydrofuran, thiophane, piperidine, 1,2,3,6-tetrahydropyridine, 1,4-dihydropyridine, piperazine, morpholine, thiomorpholine, pyran, 2,3-dihydropyran, tetrahydropyran, 1,4-dioxane, 1,3-dioxane, homopiperazine, homopiperidine, 1,3-dioxepane, 4,7-dihydro-1,3-dioxepin and hexamethyleneoxide.

Examples of heteroaryl groups include pyridyl, pyrazinyl, pyrimidinyl (such as, but not limited to, 2- and 4-pyrimidinyl), pyridazinyl, thienyl, furyl, pyrrolyl, imidazolyl, thiazolyl, oxazolyl, pyrazolyl, isothiazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,3,4-triazolyl, tetrazolyl, 1,2,3-thiadiazolyl, 1,2,3-oxadiazolyl, 1,3,4-thiadiazolyl and 1,3,4-oxadiazolyl.

Examples of polycyclic heterocycles include indolyl (such as, but not limited to, 3-, 4-, 5-, 6- and 7-indolyl), indolinyl, quinolyl, tetrahydroquinolyl, isoquinolyl (such as, but not limited to, 1- and 5-isoquinolyl), 1,2,3,4-tetrahydroisoquinolyl, cinnolinyl, quinoxalinyl (such as, but not limited to, 2- and 5-quinoxalinyl), quinazolinyl, phthalazinyl, 1,8-naphthyridinyl, 1,4-benzodioxanyl, coumarin, dihydrocoumarin, 1,5-naphthyridinyl, benzofuryl (such as, but not limited to, 3-, 4-, 5-, 6- and 7-benzofuryl), 2,3-dihydrobenzofuryl, 1,2-benzisoxazolyl, benzothienyl (such as, but not limited to, 3-, 4-, 5-, 6-, and 7-benzothienyl), benzoxazolyl, benzothiazolyl (such as, but not limited to, 2-benzothiazolyl and 5-benzothiazolyl), purinyl, benzimidazolyl, benztriazolyl, thioxanthinyl, carbazolyl, carbolinyl, acridinyl, pyrrolizidinyl, and quinolizidinyl.

The aforementioned listing of heterocyclyl and heteroaryl moieties is intended to be representative and not limiting.

As used herein, the term "substituted" means that an atom or group of atoms has replaced hydrogen as the substituent attached to another group.

For aryl, aryl-(C_1-C_3)alkyl and heterocyclyl groups, the term "substituted" as applied to the rings of these groups refers to any level of substitution, namely mono-, di-, tri-, tetra-, or penta-substitution, where such substitution is permitted. The substituents are independently selected, and substitution may be at any chemically accessible position. In one embodiment, the substituents vary in number between one and four. In another embodiment, the substituents vary in number between one and three. In yet another embodiment, the substituents vary in number between one and two. In yet another embodiment, the substituents are independently selected from the group consisting of C_{1-6} alkyl, —OH, C_{1-6} alkoxy, halo, amino, acetamido and nitro. As used herein, where a substituent is an alkyl or alkoxy group, the carbon chain may be branched, straight or cyclic, with straight being preferred.

As used herein, the term "AcOH" refers to acetic acid; the term "nBuOH" refers to n-butanol; the term "CH_2Cl_2" refers to dichloromethane (also known as methylene dichloride); the term "DMSO" refers to dimethylsulfoxide; the term "EtOAc" refers to ethyl acetate; the term "EtOH" refers to ethanol; the term "HCl" refers to hydrochloric acid or a hydrochloride salt; the term "HPLC" refers to high pressure liquid chromatography; the term "H_2SO_4" refers to sulfuric acid; the term "LCMS" refers to liquid chromatography-mass spectrometry; the term "MS" refers to mass spectrometry; the term "MeOH" refers to methanol; the term "NaCl" refers to sodium chloride; the term "$NaHCO_3$" refers to sodium bicarbonate; the term "NaOH" refers to sodium hydroxide; the term "Na_2SO_4" refers to sodium sulfate; the term "mpk" refers to mg/kg; the term "NMR" refers to nuclear magnetic resonance; the term "PE" or "pet ether" refers to petroleum ether; the term "$POCl_3$" refers to phosphorous oxychloride; the term "ppm" refers to part per million; the term "xphos" refers to 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl; the term "dba" refers to trans, trans-dibenzylideneacetone.

"Instructional material," as that term is used herein, includes a publication, a recording, a diagram, or any other medium of expression that can be used to communicate the usefulness of the composition and/or compound of the invention in a kit. The instructional material of the kit may, for example, be affixed to a container that contains the compound and/or composition of the invention or be shipped together with a container that contains the compound and/or composition. Alternatively, the instructional material may be shipped separately from the container with the intention that the recipient uses the instructional material and the compound cooperatively. Delivery of the instructional material may be, for example, by physical delivery of the publication or other medium of expression communicating the usefulness of the kit, or may alternatively be achieved by electronic transmission, for example by means of a computer, such as by electronic mail, or download from a website.

Compounds and Compositions of the Invention

The invention includes compounds of the invention, as well as any compositions comprising such compounds.

The invention includes at least one compound selected from the group consisting of: N-(2,4-bis-n-propylamino)-N-(6-methylamino)-[1,3,5]triazine (CLXXII), N-(2,4,6-tris-n-propylamino)-[1,3,5]triazine (CLXXIII), N-(2,4,6-tris-n-propylamino)-1,3-pyrimidine (CLXXIV), N-(2,4-bis-n-propylamino)-N-(6-i-propylamino)-1,3-pyrimidine (CLXXV), a salt thereof, and any combinations thereof.

In one embodiment, the salt is hydrogen sulfate, hydrochloride, phosphate, hydrogen phosphate or dihydrogen phosphate. In another embodiment, the at least one compound is formulated with at least one pharmaceutically acceptable carrier.

The invention also includes a composition comprising N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXXV) or a salt thereof, wherein 4,6-bis-(n-propylamino)-1,3,5-triazin-2-ol is present at a level equal to or lower than about 1% (w/w) with respect to (XXXV) or the salt thereof.

In one embodiment, 4,6-bis-(n-propylamino)-1,3,5-triazin-2-ol is present at a level equal to or lower than about 0.5% (w/w) with respect to (XXXV) or the salt thereof. In another embodiment, 4,6-bis-(n-propylamino)-1,3,5-triazin-2-ol is present at a level equal to or lower than about 0.3% (w/w) with respect to (XXXV) or the salt thereof. In yet another embodiment, 4,6-bis-(n-propylamino)-1,3,5-triazin-2-ol is present at a level equal to or lower than about 0.2% (w/w) with respect to (XXXV) or the salt thereof. In yet another embodiment, 4,6-bis-(n-propylamino)-1,3,5-triazin-2-ol is present at a level equal to or lower than about 0.15% (w/w) with respect to (XXXV) or the salt thereof. In yet another embodiment, 4,6-bis-(n-propylamino)-1,3,5-triazin-2-ol is present at a level equal to or lower than about 0.1% (w/w) with respect to (XXXV) or the salt thereof. In yet another embodiment, 4,6-bis-(n-propylamino)-1,3,5-triazin-2-ol is essentially absent from the composition.

The invention also includes a composition comprising N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXXV) or a salt thereof, further comprising a buffer and a liquid carrier.

In one embodiment, the salt of (XXXV) is the hydrogen sulfate salt (XXXVI). In another embodiment, the composition further comprises citric acid. In yet another embodiment, the pH of the composition is adjusted with a base. In yet another embodiment, the base comprises sodium hydroxide. In yet another embodiment, the liquid carrier comprises water. In yet another embodiment, the pH of the composition ranges from about 2.5 to about 6. In yet another embodiment, the pH of the composition ranges from about 2.5 to about 5. In yet another embodiment, the pH of the composition ranges from about 3 to about 4. In yet another embodiment, the concentration of (XXXV) or the salt thereof in the composition is about 1-10 mg/mL. In yet another embodiment, the concentration of (XXXV) or the salt thereof in the composition is about 5-10 mg/mL. In yet another embodiment, the concentration of (XXXV) or the salt thereof in the composition is about 10 mg/mL. In yet another embodiment, the composition comprises less than about 0.5% (w/w) 4,6-bis-(n-propylamino)-1,3,5-triazin-2-ol (CLXXVIII) with respect to (XXXV). In yet another embodiment, less than about 0.5% (w/w) of N-(4,6-Bis-n-propylamino)-[1,3,5]-triazin-2-ol (CLXXVIII) with respect to (XXXV) forms over at least a 6-month period of storage of the composition at 2-8° C. In yet another embodiment, less than about 0.5% (w/w) of N-(4,6-Bis-n-propylamino)-[1,3,5]-triazin-2-ol (CLXXVIII) with respect to (XXXV) forms over at least a 12-month period of storage of the composition at 2-8° C. In yet another embodiment, less than about 0.5% (w/w) of N-(4,6-Bis-n-propylamino)-[1,3,5]-triazin-2-ol (CLXXVIII) with respect to (XXXV) forms over at least an 18-month period of storage of the composition at 2-8° C. In yet another embodiment, less than about 0.5% (w/w) of N-(4,6-Bis-n-propylamino)-[1,3,5]-triazin-2-ol (CLXXVIII) with respect to (XXXV) forms over at least a 24-month period of storage of the composition at 2-8° C.

The invention also includes a crystalline free base of N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXXV), Form A, with a XRPD spectrum as per FIG. **22**.

The invention also includes at least one crystalline salt of N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N.O-dimethyl-hydroxylamine (XXXV) selected from the group consisting of:

(i) crystalline hydrogen sulfate salt (XXXVI) Form A, with a XRPD spectrum as per FIG. **14**;

(ii) crystalline hydrogen sulfate salt (XXXVI) comprising a mixture of Form A and Form B, with a XRPD spectrum as per FIG. **23**;

(iii) crystalline hydrogen sulfate salt (XXXVI) Form C, with a XRPD spectrum as per FIG. **24**;

(iv) crystalline hydrogen sulfate salt (XXXVI) Form D, with a XRPD spectrum as per FIG. **25**;

(v) crystalline hydrogen sulfate salt (XXXVI) comprising a mixture of Form A and Form D, with a XRPD spectrum as per FIG. **26**;

(vi) crystalline phosphoric acid salt (CLXXX) Form A, with a XRPD spectrum as per FIG. **27**;

(vii) crystalline phosphoric acid salt (CLXXX) Form C, with a XRPD spectrum as per FIG. **28**;

(viii) crystalline phosphoric acid salt (CLXXX) comprising a mixture of Form A and Form B, with a XRPD spectrum as per FIG. **29**;

(ix) crystalline phosphoric acid salt (CLXXX) comprising a mixture of Form C and Form D, with a XRPD spectrum as per FIG. **30**;

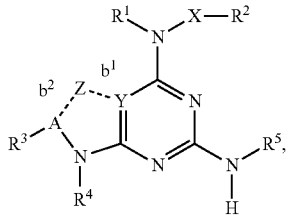
(x) crystalline phosphoric acid salt (CLXXX) comprising a mixture of Form C and Form E, with a XRPD spectrum as per FIG. **31**;

and any mixtures thereof.

The invention also includes a salt of N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-hydroxylamine (XXXV), comprising approximately one mole equivalent of sulfuric acid. In one embodiment, the salt further comprises one mole equivalent of water.

The invention also includes a salt of N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N.O-dimethyl-hydroxylamine (XXXV), comprising at least one mole equivalent of phosphoric acid. In one embodiment, the salt comprises about one mole equivalent of phosphoric acid.

The invention also includes a compound of formula (I) or a salt thereof:



(I)

wherein

R^1 and R^2 are independently H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, phenyl, substituted phenyl, phenylalkyl, substituted phenylalkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, heteroarylalkyl, substituted heteroarylalkyl, heteroaryl or substituted heteroaryl; or R^1 and R^2 combine as to form a biradical selected from the group consisting of 3-hydroxy-pentane-1,5-diyl, 6-hydroxy-cycloheptane-1,4-diyl, propane-1,3-diyl, butane-1,4-diyl and pentane-1,5-diyl;

R^3 is H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, —NR^1R^2, —C(O)OR^1, acyl, or aryl;

R^4 is H, alkyl, or substituted alkyl;

R^5 is H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, —OR^1, —NR^1R^2, —$C(O)OR^1$, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, or substituted heterocyclic; or R^3 and R^5 combine as to form a biradical selected from the group consisting of 3,6,9-trioxa-undecane-1,11-diyl and 3,6-dioxa-octane-1,8-diyl;

R^6 is H, alkyl, substituted alkyl or alkenyl;

X is a bond, O or NR^4; and,

Y is N, CR^6 or C; wherein:

if Y is N or CR^6, then bond b^1 is nil and:

(i) Z is H, bond b^2 is a single bond, and A is CH; or, (ii) Z is nil,

bond b^2 is nil, and A is a single bond; and,

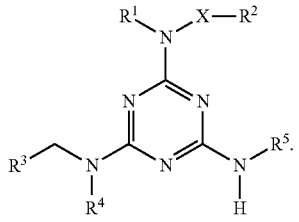
if Y is C, then bond b^1 is a single bond, and:

(i) Z is CH_2, bond b^2 is a single bond, and A is CH; or, (ii) Z is CH, bond b^2 is a double bond, and A is C.

In one embodiment, R^3 is H, alkyl, substituted alkyl, alkenyl, cycloalkyl, substituted cycloalkyl, or substituted alkenyl. In another embodiment, R^5 is H, alkyl, substituted alkyl, alkenyl, substituted alkenyl, acyl, cycloalkyl or substituted cycloalkyl.

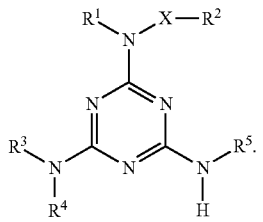
In one embodiment, Y is N, bond b^1 is nil, Z is H, bond b^2 is a single bond, A is CH, and the compound of the invention is a 1,3,5-triazine of formula (II-a) or a salt thereof:

(II-a)



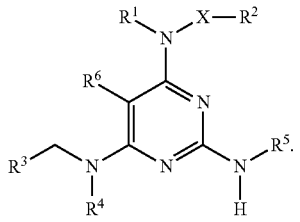
In one embodiment, Y is N, bond b^1 is nil, Z is nil, bond b^2 is nil, and A is a bond, and the compound of the invention is a 1,3,5-triazine of formula (II-b) or a salt thereof:

(II-b)



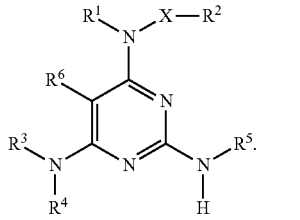
In one embodiment, Y is CR^6, bond b^1 is nil, Z is H, bond b^2 is a single bond, A is CH, and the compound of the invention is a pyrimidine of formula (III-a) or a salt thereof:

(III-a)



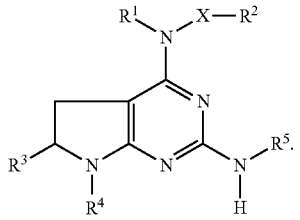
In one embodiment, Y is CR^6, bond b^1 is nil, Z is nil, bond b^2 is nil, and A is a bond, and the compound of the invention is a pyrimidine of formula (III-b) or a salt thereof:

(III-b)



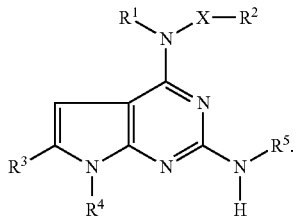
In one embodiment, Y is C, bond b^1 is a single bond, Z is CH_2, bond b^2 is a single bond, A is CH, and the compound of the invention is a pyrrolidinopyrimidine of formula (IV) or a salt thereof:

(IV)



In one embodiment, Y is C, bond b^1 is a single bond, Z is CH, bond b^2 is a double bond, A is C, and the compound of the invention is a pyrrolopyrimidine of formula (V) or a salt thereof:

(V)



In one embodiment, the compound of formula (I) is selected from the group consisting of: N-(4,6-Bis-methylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(4,6-Bis-ethylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(4-Cyclopropylmethyl)-N-(6-n-propylamino)[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(4-Ethylamino)-N-(6-n-propylamino)-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(Bis-4,6-(2-methyl-propylamino)) [1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxy-

lamine, N-(Bis-4,6-(2,2-dimethylpropylamino)) [1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(Bis-4,6-(2,2-dimethylpropylamino))[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(4-(Methoxy(methyl) amino)-6-(n-propylamino)-1,3,5-triazin-2-yl)propionamide, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-methyl-hydroxylamine, O-Allyl-N-(4,6-bis-n-propylamino-[1,3,5] triazin-2-yl)-hydroxylamine, N-(4,6-Bis-n-propylamino-[1, 3,5]triazin-2-yl)-hydroxylamine, 6-(Methoxy(methyl) amino)-N^2-propyl-1,3,5-triazine-2,4-diamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N-methyl-hydroxylamine, O-Benzyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N-methyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5] triazin-2-yl)-N-isopropyl-hydroxylamine, 6-[1,2]Oxazinan-2-yl-N,N'-dipropyl-[1,3,5]triazine-2,4-diamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isopropyl-N-methyl-hydroxylamine, O-Benzyl-N-(4,6-bis-n-propylamino-[1,3, 5]triazin-2-yl)-N-ethyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isopropyl-hydroxylamine, 6-((Benzyloxy)(isopropyl)amino)-N^2,N^4-dipropyl-1,3,5-triazine-2,4-diamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N-ethyl-O-isopropyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isobutyl-N-methyl-hydroxylamine, 6-(Methyl (thiophen-2-ylmethoxy)amino)-N^2,N^4-dipropyl-1,3,5-triazine-2,4-diamine, N-(4,6-Bis-n-propylamino-[1,3,5] triazin-2-yl)-O-cyclopropylmethyl-N-methyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-ethyl-N-methyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-(2,2-difluoro-ethyl)-hydroxylamine, 4-N-(2-Dimethylaminoethyl)amino-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, 4-N-(3-(1-N-Methylimidazol-2-yl)-propyl)-amino-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, 4-N-(1-N-Methylimidazol-2-yl)-methylamino-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N, O-dimethyl-hydroxylamine, 4,6-Bis-(N-(2-dimethylaminoethyl)amino)-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, 4,6-Bis-(N-(pyridin-4-yl-methyl) amino)-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, 4,6-Bis-[N-(3-methoxy-n-propyl)amino]-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, 4,6-Bis-[N-(tetrahydro-pyran-4-yl-methyl)amino]-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(5,8,11-Trioxa-2,14,16,18,19-pentaazabicyclo[13.3.1]nonadeca-1(18),15(19),16(17)-trien-17-yl)-N,O-dimethylhydroxylamine, 2,6-Bis-(N-n-propylamino)-[1,3]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N',N'-dimethylhydrazine, N-(4,6-Bis-n-propylamino-[1, 3,5]triazin-2-yl)-N-methyl-N'-methylhydrazine, 2-(n-Propyl)amino-4-(i-propylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine, 2-(n-Propyl)amino-4-dimethylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine, 2-(n-Propyl)amino-4-methylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine, 2-(n-Propyl)amino-4-(i-propyl)amino-7-i-propyl-pyrrolidino[2, 3-d]pyrimidine, 2,4-Bis-(n-propyl)amino-7H-pyrrolidino[2, 3-d]pyrimidine, 2-(n-Propyl)amino-4-(4-hydroxypiperidin-1-yl)-7-methyl-pyrrolidino[2,3-d]pyrimidine, 8-(7-Methyl-2-(n-propylamino)-pyrrolidino[2,3-d]pyrimidin-4-yl)-8-azabicyclo[3.2.1]octan-3-ol, N-(2-Propylamino-7H-pyrrolo [2,3d]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine, N-(2-(Propen-2-yl)amino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine, N-(2-(Propen-2-yl) amino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-O-methyl-hydroxylamine, N-(2-n-Propylamino-7-methyl-pyrrolo[2, 3d]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine, N-(2-n-Propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-O-methyl-hydroxylamine, N-(2-n-Propylamino-7-methyl-pyrrolo [2,3d]pyrimidin-4-yl)-hydrazine, N-Methyl-N-(2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine, N,N-Dimethyl-N'-(2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine, N-(2,4-Bis-n-propylamino)-N-(6-methylamino)-[1,3,5]triazine, N-(2,4,6-Tris-n-propylamino)-[1,3,5]triazine, N-(2,4,6-Tris-n-propylamino)-1,3-pyrimidine, N-(2,4-Bis-n-propylamino)-N-(6-i-propylamino)-1,3-pyrimidine, a salt thereof and mixtures thereof.

In one embodiment, the at least one compound is 2,6-bis-(N-n-propylamino)-[1,3]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine or a salt thereof. In another embodiment, the salt is hydrogen sulfate, hydrochloride, phosphate, hydrogen phosphate or dihydrogen phosphate.

In one embodiment, the at least one compound is selected from the group consisting of: 2-(n-Propyl)amino-4-(i-propylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine (CXXVI), 2-(n-Propyl)amino-4-dimethylamino-7-methyl-pyrrolidino [2,3 d]pyrimidine (CXXVIII), 2-(n-Propyl)amino-4-methylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine (CXXXI), 2-(n-Propyl)amino-4-(i-propyl)amino-7-i-propyl-pyrrolidino[2,3-d]pyrimidine (CXXXVI), 2,4-Bis-(n-propyl) amino-7H-pyrrolidino[2,3-d]pyrimidine (CXLIX), 2-(n-Propyl)amino-4-(4-hydroxypiperidin-1-yl)-7-methyl-pyrrolidino[2,3-d]pyrimidine (CLII), 8-(7-Methyl-2-(propylamino)-pyrrolidino[2,3-d]pyrimidin-4-yl)-8-azabicyclo[3.2.1]octan-3-ol (CLV), a salt thereof and mixtures thereof. In another embodiment, the salt is hydrogen sulfate, hydrochloride, phosphate, hydrogen phosphate or dihydrogen phosphate.

In one embodiment, the at least one compound is selected from the group consisting of: N-(2-Propylamino-7H-pyrrolo [2,3d]pyrimidin-4-yl)-O,N-dimethyl-hydroxylamine (CXLI), N-(2-(Propen-2-yl)amino-7-methyl-pyrrolo[2,3d] pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine (CLVIII), N-(2-(Propen-2-yl)amino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-O-methyl-hydroxylamine (CLX), N-(2-n-Propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-O,N-dimethyl-hydroxylamine (CLXII), N-(2-n-Propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-O-methyl-hydroxylamine (CLXIV), N-(2-n-Propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine (CLXVI), N-Methyl-N-(2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine (CLXVIII), N,N-dimethyl-N'-(2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine (CLXX), a salt thereof and mixtures thereof. In another embodiment, the salt is hydrogen sulfate, hydrochloride, phosphate, hydrogen phosphate or dihydrogen phosphate.

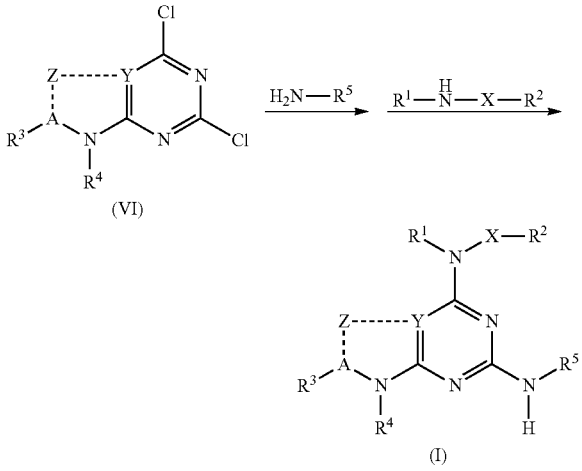
In one embodiment, the compound is formulated with at least one pharmaceutically acceptable carrier.

Preparation of the Compounds of the Invention

The compounds of the invention may be prepared according to the general methodology illustrated in the synthetic schemes described below. The reagents and conditions described herein may be modified to allow the preparation of the compounds of the invention, and such modifications are known to those skilled in the art. The scheme included herein are intended to illustrate but not limit the chemistry and methodologies that one skilled in the art may use to make compounds of the invention.

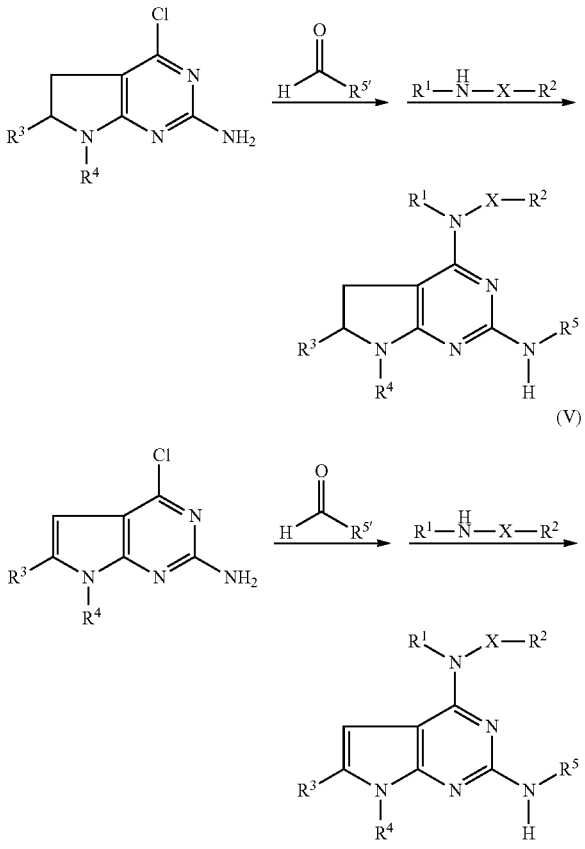
In one aspect, compounds of formula (I) may be prepared by the successive additions of (i) primary amines, (ii) a N-alkoxy-N-alkylamine or (iii) an appropriately substituted hydrazine (H_2N—NHR^2 or R^1HN—NHR^2) to suitably chlorinated intermediate (VI), as illustrated below in Scheme 1.

Scheme 1



In another aspect, a compound of formula (IV) or (V) may be prepared by reductive alkylation of a suitably chlorinated amino-pyrrolidino-pyrimidine or amino-pyrrolo-pyrimidine, respectively (Scheme 2).

Scheme 2

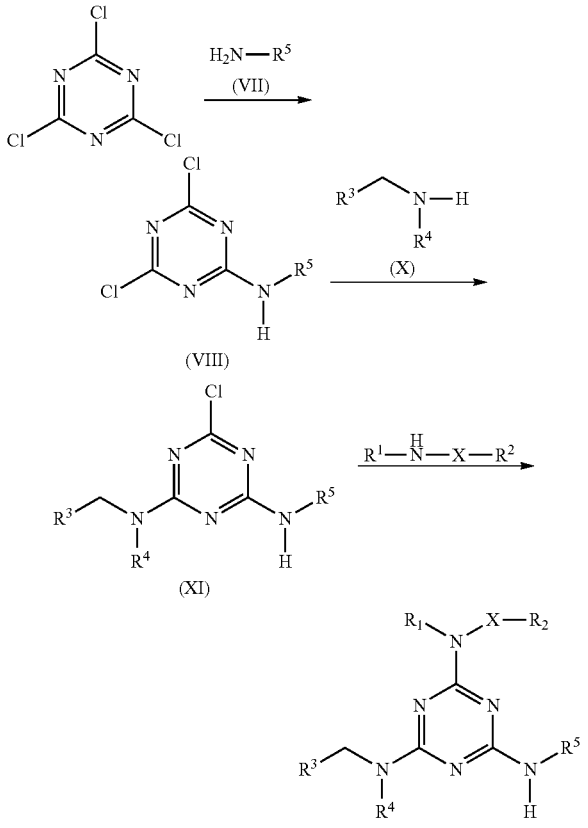


In yet another aspect, a triazine compound of formula (II) may be prepared by the successive additions of primary amines and (i) a N-alkoxy-N-alkylamine, (ii) a hydrazine $H_2N—NHR^2$, or (iii) a hydrazine $R^1HN—NHR^2$ to a suitably chlorinated triazine. Under appropriate conditions, the reaction may allow the addition of either one or two amine substituents to the triazine ring. Alternatively, first the N-alkoxy-N-alkylamine, the hydrazine $H_2N—NHR^2$, or the hydrazine $R^1HN—NHR^2$ may be added to the triazine, followed by the addition of the amines.

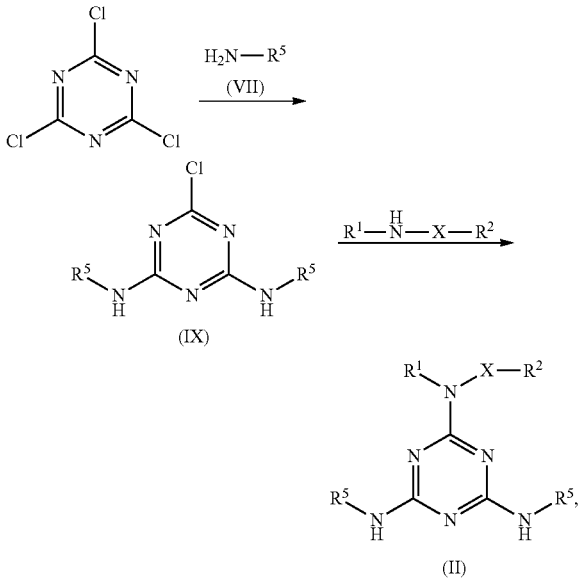
In a non-limiting example, to a solution of 2,4,6-trichlorotriazine in an appropriate aprotic or protic solvent containing an inorganic or organic base, is added a solution of a primary amine (VII) and the reaction is allowed to proceed at ambient temperature or heated, to isolate mono-amine adduct (VIII) or bis-amine adduct (IX).

In a subsequent reaction, mono-amine adduct (VIII) is reacted with another primary amine or a secondary amine (X) to yield the unsymmetrical monochloro-bis-amino-triazine adduct (XI). In a subsequent reaction, monochloro-bis-amino-triazine adduct (XI) is reacted with (i) a N-alkoxy-N-alkylamine, (ii) a hydrazine $H_2N—NHR^2$ or (iii) a hydrazine $R^1HN—NHR^2$ in an appropriate aprotic or protic solvent containing an inorganic or organic base to produce desired compounds of formula (II) (Scheme 3).

Alternatively, in a subsequent reaction, bis-amine adduct (IX) is reacted with (i) a N-alkoxy-N-alkylamine, (ii) a hydrazine $H_2N—NHR^2$ or (iii) a hydrazine $R^1HN—NHR^2$ in an appropriate aprotic or protic solvent containing an inorganic or organic base to produce desired compounds of formula (II), wherein R^3CH_2 is R^5 (Scheme 4).



Scheme 4

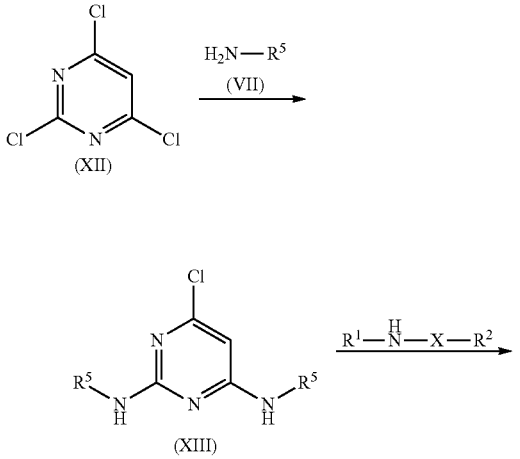


wherein R^3CH_2 is R^5

In yet another aspect, the pyrimidine compound of the formula (III) may be prepared by the successive additions of primary amines and (i) a N-alkoxy-N-alkylamine, (ii) a hydrazine $H_2N—NHR^2$ or (iii) a hydrazine $R^1HN—NHR^2$ to a suitably chlorinated pyrimidine.

In a non-limiting example, to a solution of 2,4,6-trichloropyrimidine (XII) in an appropriate aprotic or protic solvent containing an inorganic or organic base is added a solution of a primary amine (VII) and the reaction is allowed to proceed at ambient temperature or heated, yielding bis-amine adduct (XIII). In a subsequent reaction, bis-amine adduct (XIII) is reacted with (i) a N-alkoxy-N-alkylamine, (ii) a hydrazine $H_2N—NHR^2$, or (iii) a hydrazine $R^1HN—NHR^2$ in an appropriate aprotic or protic solvent containing an inorganic or organic base to produce desired compounds of formula (III) (Scheme 5).

Scheme 5



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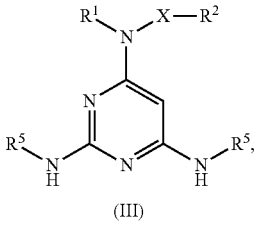
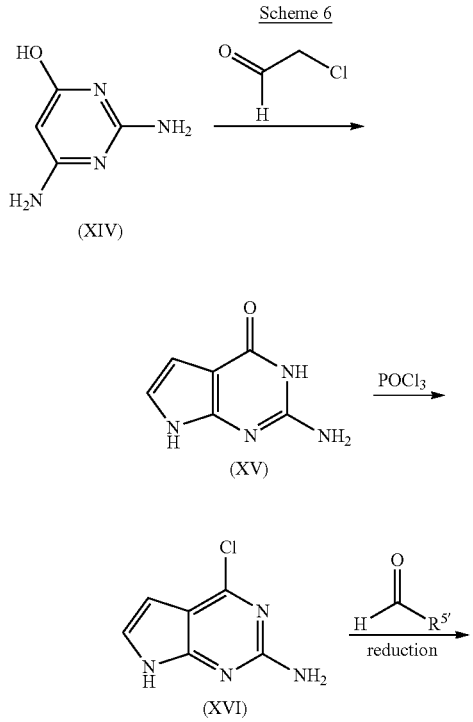


R^3CH_2 is R^5

In yet another aspect, a pyrrolidino-pyrimidine of formula (IV) or a pyrrolo-pyrimidine compounds of formula (V) may be prepared from an appropriately chlorinated aminopyrrolidinopyrimidine or aminopyrrolopyrimidine intermediate, respectively.

In a non-limiting example, 2-chloroacetaldehyde may be added to a solution of 2,6-diamino-4-hydroxy-1,3-pyrimidine (XIV) in a polar protic solvent, at ambient temperature or under heating, to yield cyclized adduct (XV). Subsequent treatment with a chlorinating agent, such as, but not limited to, phosphorous oxychloride produces the chloro intermediate (XVI). Intermediate (XVI) may be submitted to reductive alkylation with an aldehyde in the presence of a reducing agent, such as a borohydride (in a non-limiting example, cyanoborohydride) in a protic solvent, at ambient temperature or elevated temperature, to produce the amino substituted adduct (XVII). In a subsequent reaction, amino substituted adduct (XVII) is reacted with (i) a N-alkoxy-N-alkylamine, (ii) a hydrazine $H_2N—NHR^2$, or (iii) a hydrazine $R^1HN—NHR^2$ in an appropriate aprotic or protic solvent containing an inorganic or organic base to produce desired compounds of formula (V), wherein R^3 and R^4 are H (Scheme 6).

Scheme 6



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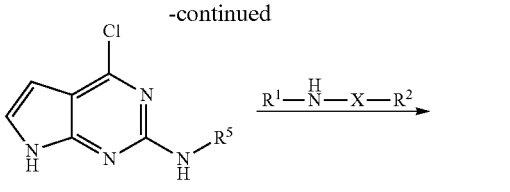
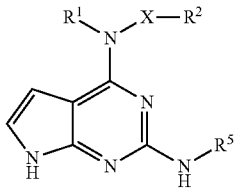


(XVII)

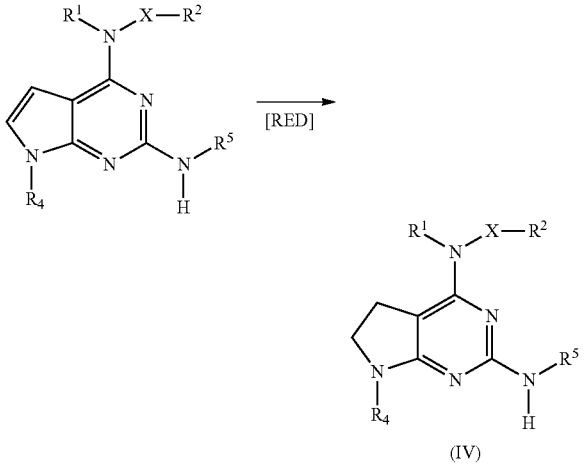


(V)

R^3 and R^4 are H

In a non-limiting example, a pyrrolidinopyrimidine compound of the formula (IV) may be prepared from the corresponding pyrrolopyrimidine analog via reduction (Scheme 7).

Scheme 7



(IV)

The manufacture of active pharmaceutical ingredients (APIs) may introduce or generate impurities in an intermediate or final active pharmaceutical compound. These impurities may include trace metals, processing aide residuals (e.g., silica, cellulose fibers), or solvents derived from purchased starting materials. Other impurities may form as a consequence of the conditions to which the starting materials, process intermediates, or active ingredient itself are exposed. These impurities may be problematic, affecting the manufacturing process as a whole and the active ingredient in particular (e.g., its purity and crystallinity, and conditions for its isolation or crystallization). Such impurities often carry forward into the isolated API.

Process-related impurities derived from degradation or side reactions involving the starting materials, intermediates, reactants, solvents or APIs are often related to the structure of the API itself. Regulatory guidances (e.g., IQC Q3b R3) provide accepted limits to which such potentially structurally related species may be present in the active ingredient, and provide threshold levels above which such impurities should be identified, or both identified and qualified with toxicology data. In some instances, a given impurity may be present within acceptable levels and still affect the behavior of the active ingredient as a drug product formulated for exploratory or approved medical use. For example, a process impurity present within acceptable levels in the active ingredient from a regulatory perspective may form an unacceptable precipitate in preparations for parenteral use.

Under certain conditions where the compounds of the present invention and their synthetic intermediates are exposed to acidic or alkaline aqueous environments, the bond between the aromatic heterocycle and the ring substituents may be susceptible to hydrolytic cleavage. For example, in the intermediates to compounds of formula (II-a), a halo substituent in the 2 position (Schemes 3 and 4) may be hydrolytically replaced with a hydroxyl group to yield N-(4,6-Bis (n-propylamino)-1,3,5-triazin-2-ol (CLXXVIII). Similarly, the amine substituents of compounds of formula (II-a) may be displaced by a hydroxyl group. Without wishing to be bound by theory, a series of chemical changes may occur to enable this substitution, such as a prior oxidation of the appended amine side chain, with hydrolytic replacement by hydroxyl as the final transformation. As a specific non-limiting example, the N,O-dimethylhydroxylamino substituent of N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXXV) may be partially displaced by hydroxyl during processing in the presence of water, forming (CLXXVIII) as a process impurity. (CLXXVIII) is unreported, has very low solubility in most organic solvents and is sparingly soluble in aqueous environments. It was unexpectedly observed that even at elevated temperatures (CLXXVIII) is very insoluble relative to the active ingredient. Thus, trace quantities of this compound in the API can be problematic in liquid formulations. In one embodiment, (CLXXVIII) formed during processing is removed from the system by filtering a solution of the active ingredient as the free base at an elevated temperature. Remaining levels of the impurity present after such filtration may be purged during solid product isolation in the mother liquor and final product washes. In one embodiment, the level of (CLXXVIII) in the isolated (XXXVI) is less that 1% (w/w) with respect to (XXXVI). In yet another embodiment, the level of (CLXXVIII) in the isolated (XXXVI) is less that 0.5% (w/w) with respect to (XXXVI). In yet another embodiment, the level of (CLXXVIII) in the isolated (XXXVI) is less that 0.3% (w/w) with respect to (XXXVI). In yet another embodiment, the level of (CLXXVIII) in the isolated (XXXVI) is less that 0.2% (w/w) with respect to (XXXVI). In yet another embodiment, the level of (CLXXVIII) in the isolated (XXXVI) is less that 0.15% (w/w) with respect to (XXXVI). In yet another embodiment, the level of (CLXXVIII) in the isolated (XXXVI) is less that 0.1% (w/w) with respect to (XXXVI). In yet another embodiment, the isolated (XXXVI) is essentially free of 4,6-bis(n-propylamino)-1,3,5-triazin-2-ol (CLXXVIII).

Formulation and Drug Product Stability

Extensive experimentation was conducted to devise a suitable liquid formulation for (XXXVI). Experimentation had shown the pKa of (XXXVI) to be ca. 5.6. A pH solubility study was performed to assess the feasibility of formulating (XXXVI) as a simple buffer solution at a reasonable pH for an IV formulation. Solubility was assessed for (XXXV) alone (FIG. **19**A) or when admixed with citric acid and adjusted to pH 3-5.5 with 6N sodium hydroxide (FIG. **19**B). From the resulting data (Table 12A, FIG. **19**B), solubility of (XXXV) was calculated to be 10 mg/mL at ca. pH 3.86, 25 mg/mL at ca. pH 3.46, and 50 mg/mL at ca. pH 3.16. The solubility

studies in water suggested solubility might be inadequate above a pH of ca. 3.6 to enable useful concentrations. Formulation at pH<3.6 may be tolerable when the formulated drug is diluted prior to administration, however the drug stability could be an issue at such a low pH.

To further evaluate the possibility for an all-aqueous formulation, stability studies of (XXXV) as a function of pH were undertaken in the presence of a buffer, with special attention to drug and buffer concentration to minimize drug precipitation and avoid buffer-catalyzed drug degradation (FIG. **20**A, Table 12B). (XXXVI) in water (1 mg/mL) was admixed with a solution of citric acid and glycine (10 mM each), the mix adjusted to pH 2-5, and exposed to 40° C., 60° C., 25° C. (control) and −70° C. (control) and evaluated for physical appearance, pH, assay and related substances after 1 week, 2 weeks and 4 weeks of storage. At 40° C. and 60° C., degradant levels at pH 2 were ca. 10-fold higher vs. pH 3-6, however at pH 3-6 growth in impurities to levels >0.2 was generally still observed. Due to the limited solubility of (XXXV) above pH 3.6, the stability was evaluated in the pH range 2-3.6 using methodology similar to that used for pH 2-5 (FIG. **20**B, Table 12C). Assay and sample pH showed little change, however impurities growth occurred across the range, with impurity levels at pH 2≥10 fold higher than impurity levels at pH 3.6.

As another option, formulations including a co-solvent matrix could be useful in providing good solubility for the salt and free base forms at a suitable pH. The solubilities of (XXXV) and (XXXVI) were evaluated in various solvents and standard IV solutions, including 0.9% NaCl, 5% dextrose, ethanol, methanol, acetonitrile, 30% PEG 400, 70% PEG 400 and 100% PEG 400, 5% Tween 80, and propylene glycol (Table 12D). The solubility of (XXXVI) exceeded 90 mg/mL in water (>400 mg/mL), IV solutions (0.9% saline and 5% dextrose), ethanol and methanol, and in 30%, 70% and 100% propylene glycol. Its solubility in 100% PEG-400 was ca. 36 mg/mL but ≥90 mg/mL in 30% and 70% PEG-400. The saturation solubility of (XXXV) in water was ca. 0.21 mg/mL, which agreed with the predicted value from the pH solubility study. The solubility in ethanol, methanol and acetonitrile were >90 mg/mL. The solubility in PEG-400 and propylene glycol systems improved with increasing levels of the cosolvent.

The impact of buffer strength on stability was studied using admixtures of (XXXV) with 10-250 mmole citric acid at pH 3 (Table 12F). Impurity levels increased noticeably at the highest buffer concentration, but there was no effect of buffer strength at 40° C. within the range of buffer concentration relevant to the formulation (ca. 10-50 mM).

A total of nine (XXXVI) formulation prototypes were prepared, including a simple buffered solution, co-solvent systems as well as non-aqueous solutions (Table 12G). The solutions were prepared at 25 mg/mL (XXXV) as free base, corresponding to 35 mg/mL (XXXVI). Prototypes 6, 7 and 8 resulted in significant precipitation during preparation and hence were not evaluated further. The remaining prototypes were stored at 2-8° C., room temperature, 40° C. and 60° C. in serum vials. Samples stored at 2-8° C. and RT were analyzed at selected time points for stability (Tables 12G.1-12G.6).

The study suggested that a buffered aqueous formulation of (XXXV) near pH 3 would be stable under refrigerated or 25° C. conditions (Table 12G.1). Based on the data used to prepare FIG. **19**B, reducing the (XXXV) concentration by half would allow the formulation pH to be increased by about 0.3 units, and as can be seen in Table 12C, a 0.3 unit pH increase substantially improved stability of the formulation. Accordingly, the concentration of (XXXV) in the formulation as per Table 12G, line 1, was reduced from 25 mg/mL to 10 mg/mL, the pH increased from 3.0 to 3.2, and the buffer concentration was proportionately decreased from 50 mM to 20 mM. The final formulation is provided in Table 12H.

A batch of (XXXVI) formulated for clinical use was placed under formal GMP stability (Tables 20I.1 and 12I.2). The data obtained indicate that a single degradant was formed over time. The degradant was identified as N-(4,6-Bis(propylamino)-1,3,5-triazin-2-ol (CLXXVIII). The result of the GMP stability study indicated the degradant can be expected to remain below 0.5% for at least two years (FIG. **21**, Table 12J).

In one aspect, when compounds of the invention are partly or entirely dissolved in water or aqueous mixtures comprising suitable organic solvents, hydrolytic degradants may be observed over time. For example, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXXV) may degrade to 4,6-Bis(propylamino)-1,3,5-triazin-2-ol (CLXXVIII) in water-based formulations. Degradation was increasingly more pronounced as the pH was reduced below a value of 3 (FIGS. **20**A and **20**B). For (XXXV) solubility was high at pH 3.5 or lower, and decreased rapidly at ca. pH 3.5 to ca. 4.4 (FIGS. **19**A and **19**B).

In another aspect, the pH of the mixture containing (XXXV) is adjusted to a specific range to balance solubility and hydrolytic stability at any temperature selected. The rate of degradation for (XXXV) was highest in the pH range where (XXXV) is soluble to clinically useful levels. In one embodiment, the clinically useful concentration of (XXXV) in a liquid carrier is about 1-10 mg/mL. In another embodiment, the clinically useful concentration of (XXXV) in a liquid carrier is about 5-10 mg/mL. Degradation of (XXXV) in an aqueous environment was also highly temperature-dependent, wherein cold temperatures slowed degradation.

In one embodiment, (XXXV) as its hydrogen sulfate salt (XXXVI) in a liquid carrier is admixed with agents to adjust pH to optimize solubility and minimize degradation. In another embodiment, (XXXV) as its hydrogen sulfate salt (XXXVI) in a liquid carrier is admixed with a buffering aid. Suitable buffering aids include, but are not limited to citric acid, ascorbic acid, monobasic phosphoric acid, and lactic acid. In an embodiment, the buffering aid is citric acid. In another embodiment, (XXXV) as its hydrogen sulfate salt (XXXVI) is admixed with citric acid and a base to adjust pH as necessary to optimize solubility and minimize hydrolytic degradation. Suitable bases include, but are not limited to, sodium hydroxide, potassium hydroxide, ammonium hydroxide, and dibasic potassium hydrogen phosphate. In one embodiment, the pH is adjusted with sodium hydroxide. In another embodiment, the liquid carrier is a mixture of water and a suitable organic co-solvent. Suitable organic co-solvents include, but are not limited to, glycerin, propylene glycol, polyethylene glycol 400, ethanol, 1,4-butanediol, and dimethylsulfoxide. In another embodiment, the liquid carrier is water.

In one embodiment, the mixture comprising (XXXV) as its sulfate salt (XXXVI) with citric acid and sodium hydroxide in water is adjusted to a pH of about 2.5 to 6. In another embodiment, the mixture is adjusted to a pH of about 3 to 5. In yet another embodiment, the mixture is adjusted to a pH of about 3 to 4.

In one embodiment, the mixture comprising (XXXV) as it hydrogen sulfate salt may be stored at low temperatures to retard the rate of degradation of the compound. In another embodiment, the temperature may be in the range of about 0-15° C. In yet another embodiment, the cold temperature

may be in the range of 2-8° C. In yet another embodiment, the mixture comprising (XXXV) as its hydrogen sulfate salt (XXXVI) and citric acid and sodium hydroxide is adjusted to pH of about 3-4 and stored at about 2-8° C. The rate of degradant growth was slow at 2-8° C. and much faster at 25° C. or above. As illustrated in FIG. **21**, the level of (CLXX-VIII) formed when (XXXVI) is formulated for parenteral administration (non-limiting conditions: aqueous media at pH 3-4) may be maintained below 0.5% for over 2 years by storing the preparation at 2-8° C. (FIG. **21**).

The present invention includes a formulation comprising an active ingredient, salt former, admixed buffering aid, admixed base pH and liquid carrier, along with storage conditions necessary to optimize drug solubility and maintain hydrolytic degradants within acceptable levels.

Salts

The compounds described herein may form salts with acids, and such salts are included in the present invention. In one embodiment, the salts are pharmaceutically acceptable salts. The term "salts" embraces addition salts of free acids that are useful within the methods of the invention. The term "pharmaceutically acceptable salt" refers to salts that possess toxicity profiles within a range that affords utility in pharmaceutical applications. Pharmaceutically unacceptable salts may nonetheless possess properties such as high crystallinity, which have utility in the practice of the present invention, such as for example utility in process of synthesis, purification or formulation of compounds useful within the methods of the invention.

Suitable pharmaceutically acceptable acid addition salts may be prepared from an inorganic acid or from an organic acid. Examples of inorganic acids include sulfate, hydrogen sulfate, hydrochloric, hydrobromic, hydriodic, nitric, carbonic, sulfuric, and phosphoric acids (including hydrogen phosphate and dihydrogen phosphate). Appropriate organic acids may be selected from aliphatic, cycloaliphatic, aromatic, araliphatic, heterocyclic, carboxylic and sulfonic classes of organic acids, examples of which include formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucuronic, maleic, fumaric, pyruvic, aspartic, glutamic, benzoic, anthranilic, 4-hydroxybenzoic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, benzenesulfonic, pantothenic, trifluoromethanesulfonic, 2-hydroxyethanesulfonic, p-toluenesulfonic, sulfanilic, cyclohexylaminosulfonic, stearic, alginic, β-hydroxybutyric, salicylic, galactaric and galacturonic acid.

Suitable pharmaceutically acceptable base addition salts of compounds of the invention include, for example, metallic salts including alkali metal, alkaline earth metal and transition metal salts such as, for example, calcium, magnesium, potassium, sodium and zinc salts. Pharmaceutically acceptable base addition salts also include organic salts made from basic amines such as, for example, N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine. All of these salts may be prepared from the corresponding compound by reacting, for example, the appropriate acid or base with the compound.

Combination Therapies

In one embodiment, the compounds of the invention are useful in the methods of present invention in combination with at least one additional compound useful for treating breathing control disorders. These additional compounds may comprise compounds of the present invention or other compounds, such as commercially available compounds, known to treat, prevent, or reduce the symptoms of breathing disorders. In embodiment, the combination of at least one compound of the invention or a salt thereof and at least one additional compound useful for treating breathing disorders has additive, complementary or synergistic effects in the treatment of disordered breathing, and in the treatment of sleep-related breathing disorders.

In a non-limiting example, the compounds of the invention or a salt thereof may be used in combination with one or more of the following drugs: acetazolamide, almitrine, theophylline, caffeine, methylprogesterone and related compounds, serotinergic modulators, cannabinoids (such as but not limited to dronabinol), and compounds known as ampakines Non-limiting examples of ampakines are the pyrrolidine derivative racetam drugs such as piracetam and aniracetam; the "CX" series of drugs which encompass a range of benzoylpiperidine and benzoylpyrrolidine structures, such as CX-516 (6-(piperidin-1-yl-carbonyl)quinoxaline), CX-546 (2,3-dihydro-1,4-benzodioxin-7-yl-(1-piperidyl)-methanone), CX-614 (2H,3H,6aH-pyrrolidino(2,1-3',2')-1,3-oxazino-(6',5'-5,4)benzo(e)1,4-dioxan-10-one), CX-691 (2,1,3-benzoxadiazol-6-yl-piperidin-1-yl-methanone), CX-717, CX-701, CX-1739, CX-1763, and CX-1837; benzothiazide derivatives such as cyclothiazide and IDRA-21 (7-chloro-3-methyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide); biarylpropylsulfonamides such as LY-392,098, LY-404,187 (N-[2-(4'-cyanobiphenyl-4-yl)propyl]propane-2-sulfonamide), LY-451,646 and LY-503,430 (4'-{(1S)-1-fluoro-2-[(isopropylsulfonyl)amino]-1-methylethyl}-N-methylbiphenyl-4-carboxamide).

A synergistic effect may be calculated, for example, using suitable methods such as, for example, the Sigmoid-E_{max} equation (Holford & Scheiner, 19981, Clin. Pharmacokinet 6: 429-453), the equation of Loewe additivity (Loewe & Muischnek, 1926, Arch. Exp. Pathol Pharmacol. 114: 313-326) and the median-effect equation (Chou & Talalay, 1984, Adv. Enzyme Regul. 22: 27-55). Each equation referred to above may be applied to experimental data to generate a corresponding graph to aid in assessing the effects of the drug combination. The corresponding graphs associated with the equations referred to above are the concentration-effect curve, isobologram curve and combination index curve, respectively.

Methods of the Invention

The invention includes a method of preventing or treating a breathing control disorder or disease in a subject in need thereof. The method comprises administering to the subject an effective amount of a pharmaceutical formulation comprising at least one pharmaceutically acceptable carrier and at least one compound selected from the group consisting of: N-(2,4-bis-n-propylamino)-N-(6-methylamino)-[1,3,5]triazine (CLXXII), N-(2,4,6-tris-n-propylamino)-[1,3,5]triazine (CLXXIII), N-(2,4,6-tris-n-propylamino)-1,3-pyrimidine (CLXXIV), N-(2,4-bis-n-propylamino)-N-(6-i-propylamino)-1,3-pyrimidine (CLXXV), a salt thereof, and any combinations thereof.

The invention also includes a method of preventing destabilization or stabilizing breathing rhythm in a subject in need thereof. The method comprises administering to the subject an effective amount of a pharmaceutical formulation comprising at least one pharmaceutically acceptable carrier and at least one compound selected from the group consisting of: N-(2,4-bis-n-propylamino)-N-(6-methylamino)-[1,3,5]triazine (CLXXII), N-(2,4,6-tris-n-propylamino)-[1,3,5]triazine (CLXXIII), N-(2,4,6-tris-n-propylamino)-1,3-pyrimidine (CLXXIV), N-(2,4-bis-n-propylamino)-N-(6-i-propylamino)-1,3-pyrimidine (CLXXV), a salt thereof, and any combinations thereof.

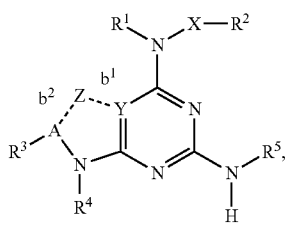
In one embodiment, the breathing control disorder or disease is selected from the group consisting of respiratory depression, sleep apnea, apnea of prematurity, obesity-hypoventilation syndrome, primary alveolar hypoventilation syndrome, dyspnea, hypoxia, and hypercapnia. In another embodiment, the respiratory depression is caused by an anesthetic, a sedative, an anxiolytic agent, a hypnotic agent, alcohol or a narcotic. In yet another embodiment, the subject is further administered a composition comprising at least one additional compound useful for treating the breathing control disorder or disease. In yet another embodiment, the at least one additional compound is selected from the group consisting of acetazolamide, almitrine, theophylline, caffeine, methyl progesterone, a serotinergic modulator, a cannabinoid and an ampakine. In yet another embodiment, the formulation is administered in conjunction with the use of a mechanical ventilation device or positive airway pressure device on the subject. In yet another embodiment, the subject is a mammal. In yet another embodiment, the mammal is a human. In yet another embodiment, the formulation is administered to the subject by an inhalational, topical, oral, buccal, rectal, vaginal, intramuscular, subcutaneous, transdermal, intrathecal or intravenous route.

The invention also includes a method of reducing or minimizing the open channel fraction of the potassium maxi-K or BK channel in a subject in need thereof. The method comprises administering to the subject an effective amount of a pharmaceutical formulation comprising at least one pharmaceutically acceptable carrier and at least one compound of formula (I).

The invention also includes a method of inhibiting the TASK-1 (KCNK3) channel in a subject in need thereof. The method comprises administering to the subject an effective amount of a pharmaceutical formulation comprising at least one pharmaceutically acceptable carrier and at least one compound of formula (I).

The invention also includes a method of increasing minute ventilation in a subject in need thereof. The method comprises administering to the subject an effective amount of a pharmaceutical formulation comprising at least one pharmaceutically acceptable carrier and at least one compound of formula (I).

In one embodiment, the compound of formula (I) is:



(I)

wherein

R^1 and R^2 are independently H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, phenyl, substituted phenyl, phenylalkyl, substituted phenylalkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, heteroarylalkyl, substituted heteroarylalkyl, heteroaryl or substituted heteroaryl; or R^1 and R^2 combine as to form a biradical selected from the group consisting of 3-hydroxy-pentane-1,5-diyl, 6-hydroxy-cycloheptane-1,4-diyl, propane-1,3-diyl, butane-1,4-diyl and pentane-1,5-diyl;

R^3 is H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, —NR^1R^2, —$C(O)OR^1$, acyl, or aryl;

R^4 is H, alkyl, or substituted alkyl;

R^5 is H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, —OR^1, —NR^1R^2, —$C(O)OR^1$, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, or substituted heterocyclic; or R^3 and R^5 combine as to form a biradical selected from the group consisting of 3,6,9-trioxa-undecane-1,11-diyl and 3,6-dioxa-octane-1,8-diyl;

R^6 is H, alkyl, substituted alkyl or alkenyl;

X is a bond, O or NR^4; and,

Y is N, CR^6 or C; wherein:

if Y is N or CR^6, then bond b^1 is nil and:

(i) Z is H, bond b^2 is a single bond, and A is CH; or,

(ii) Z is nil, bond b^2 is nil, and A is a single bond; and,

if Y is C, then bond b^1 is a single bond, and:

(i) Z is CH_2, bond b^2 is a single bond, and A is CH; or,

(ii) Z is CH, bond b^2 is a double bond, and A is C;

or a salt thereof.

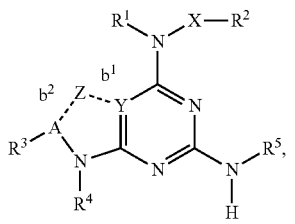
In another embodiment, the compound of formula (I) is selected from the group consisting of: N-(4,6-Bis-methylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(4,6-Bis-ethylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(4-Cyclopropylmethyl)-N-(6-n-propylamino)[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(4-Ethylamino)-N-(6-n-propylamino)-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(Bis-4,6-(2-methylpropylamino)) [1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(Bis-4,6-(2,2-dimethylpropylamino)) [1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(Bis-4,6-(2,2-dimethylpropylamino))[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(4-(Methoxy(methyl)amino)-6-(n-propylamino)-1,3,5-triazin-2-yl)propionamide, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-methyl-hydroxylamine, O-Allyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-hydroxylamine, 6-(Methoxy(methyl)amino)-N^2-propyl-1,3,5-triazine-2,4-diamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N-methyl-hydroxylamine, O-Benzyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N-methyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N-isopropyl-hydroxylamine, 6-[1,2]Oxazinan-2-yl-N,N'-dipropyl-[1,3,5]triazine-2,4-diamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isopropyl-N-methyl-hydroxylamine, O-Benzyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N-ethyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isopropyl-hydroxylamine, 6-((Benzyloxy)(isopropyl)amino)-N^2,N^4-dipropyl-1,3,5-triazine-2,4-diamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N-ethyl-O-isopropyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isobutyl-N-methyl-hydroxylamine, 6-(Methyl(thiophen-2-ylmethoxy)amino)-N^2,N^4-dipropyl-1,3,5-triazine-2,4-diamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-cyclopropylmethyl-N-methyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-ethyl-N-methyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-(2,2-difluoro-ethyl)-hydroxylamine, 4-N-(2-Dimethylaminoethyl)amino-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, 4-N-(3-(1-N-Methylimidazol-2-yl)-propyl)-amino-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, 4-N-(1-N-Methylimidazol-2-yl)-methylamino-6- N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,

O-dimethyl-hydroxylamine, 4,6-Bis-(N-(2-dimethylamino-ethyl)amino)-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxy-lamine, 4,6-Bis-(N-(pyridin-4-yl-methyl)amino)-[1,3,5] triazin-2-yl)-N,O-dimethyl-hydroxylamine, 4,6-Bis-[N-(3-methoxy-n-propyl)amino]-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, 4,6-Bis-[N-(tetrahydropyran-4-yl-methyl)amino]-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(5,8,11-Trioxa-2,14,16,18,19-pentaazabicyclo[13.3.1]nonadeca-1(18),15(19),16(17)-trien-17-yl)-N,O-dimethylhydroxylamine, 2,6-Bis-(N-n-propylamino)-[1,3]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N',N'-dimethylhydrazine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N-methyl-N'-methylhydrazine, 2-(n-Propyl)amino-4-(1-propylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine, 2-(n-Propyl)amino-4-dimethylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine, 2-(n-Propyl)amino-4-methylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine, 2-(n-Propyl)amino-4-(i-propyl)amino-7H-propyl-pyrrolidino[2,3-d]pyrimidine, 2,4-Bis-(n-propyl)amino-7H-pyrrolidino[2,3-d]pyrimidine, 2-(n-Propyl)amino-4-(4 hydroxypiperidin-1-yl)-7-methyl-pyrrolidino[2,3-d]pyrimidine, 8-(7-Methyl-2-(n-propylamino)-pyrrolidino[2,3-d]pyrimidin-4-yl)-8-azabicyclo[3.2.1]octan-3-ol, N-(2-Propylamino-7H-pyrrolo[2,3d]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine, N-(2-(Propen-2-yl)amino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine, N-(2-(Propen-2-yl) amino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-O-methyl-hydroxylamine, N-(2-n-Propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine, N-(2-n-Propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-O-methyl-hydroxylamine, N-(2-n-Propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine, N-Methyl-N-(2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine, N,N-Dimethyl-N'-(2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine, N-(2,4-Bis-n-propylamino)-N-(6-methylamino)-[1,3,5]triazine, N-(2,4,6-Tris-n-propylamino)-[1,3,5]triazine, N-(2,4,6-Tris-n-propylamino)-1,3-pyrimidine, N-(2,4-Bis-n-propylamino)-N-(6-i-propylamino)-1,3-pyrimidine, a salt thereof and mixtures thereof. In another embodiment, the compound of formula (I) is selected from the group consisting of: N-(4,6-Bis-n-propylamino-1,3,5]triazin-2-yl)-O-methyl-hydroxy-lamine; N-(4,6-Bis-n-propylamino-1,3,5]triazin-2-yl)-N',N'-dimethylhydrazine; N-(2,4-Bis-n-propylamino)-N-(6-methylamino)-[1,3,5]triazine; N-[(2,6-Bis-n-propylamino)-pyrimidin-4-yl]-N,O-dimethyl-hydroxylamine; N,N-Dimethyl-N'-(2-n-propylamino-7-methyl-pyrrolo[2,3d] pyrimidin-4-yl)-hydrazine; a salt thereof and mixtures thereof. In yet another embodiment,

In one embodiment, the subject is a mammal. In another embodiment, the mammal is human. the compound of formula (I) is N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, or a salt thereof.

In one embodiment, the pharmaceutical formulation is administered via intravenous infusion. In another embodiment, the infusion dose of the pharmaceutical formulation is at least about 0.016 mg/kg/min. In yet another embodiment, the infusion dose of the pharmaceutical formulation is about 0.016 mg/kg/min. In yet another embodiment, the infusion dose of the pharmaceutical composition produces plasma concentrations of at least about 726 ng/mL in the subject. In yet another embodiment, the infusion dose of the pharmaceutical composition produces plasma concentrations of at least about 726 ng/mL in a mammal.

The present invention also includes a method of preventing or treating a breathing control disorder or disease in a subject in need thereof. The method includes administering to the subject an effective amount of a pharmaceutical formulation comprising at least a pharmaceutically acceptable carrier and at least one compound of formula (I):



(I)

R^1 and R^2 are independently H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, phenyl, substituted phenyl, phenylalkyl, substituted phenylalkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, heteroarylalkyl, substituted heteroarylalkyl, heteroaryl or substituted heteroaryl; or R^1 and R^2 combine as to form a biradical selected from the group consisting of 3-hydroxy-pentane-1,5-diyl, 6-hydroxy-cycloheptane-1,4-diyl, propane-1,3-diyl, butane-1,4-diyl and pentane-1,5-diyl;

R^3 is H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, —NR^1R^2, —$C(O)OR^1$, acyl, or aryl;

R^4 is H, alkyl, or substituted alkyl;

R^5 is H, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, —OR^1, —NR^1R^2, —$C(O)OR^1$, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, or substituted heterocyclic; or R^3 and R^5 combine as to form a biradical selected from the group consisting of 3,6,9-trioxa-undecane-1,11-diyl and 3,6-dioxa-octane-1,8-diyl;

R^6 is H, alkyl, substituted alkyl or alkenyl;

X is a bond, O or NR^4; and,

Y is N, CR^6 or C; wherein:

if Y is N or CR^6, then bond b^1 is nil and:

(i) Z is H, bond b^2 is a single bond, and A is CH; or, (ii) Z is nil, bond b^2 is nil, and A is a single bond; and,

if Y is C, then bond b^1 is a single bond, and:

(i) Z is CH_2, bond b^2 is a single bond, and A is CH; or, (ii) Z is CH, bond b^2 is a double bond, and A is C;

or a salt thereof.

The present invention also includes a method of preventing destabilization of or stabilizing breathing rhythm in a subject in need thereof. The method includes administering to the subject an effective amount of a pharmaceutical formulation comprising at least a pharmaceutically acceptable carrier and at least one compound of formula (I) or a salt thereof.

In one embodiment, administering the formulation of the invention stabilizes the breathing rhythm of the subject. In another embodiment, administering the formulation of the invention increases minute ventilation in the subject.

In one embodiment, the destabilization is associated with a breathing control disorder or disease.

In one embodiment, the breathing disorder or disease is selected from the group consisting of narcotic-induced respiratory depression, anesthetic-induced respiratory depression, sedative-induced respiratory depression, anxiolytic-induced respiratory depression, hypnotic-induced respiratory depression, alcohol-induced respiratory depression, analgesic-induced respiratory depression, sleep apnea, apnea of prematurity, obesity-hypoventilation syndrome, primary alveolar

hypoventilation syndrome, dyspnea, altitude sickness, hypoxia, hypercapnia and chronic obstructive pulmonary disease (COPD). In another embodiment, the respiratory depression is caused by an anesthetic, a sedative, an anxiolytic agent, a hypnotic agent, alcohol or a narcotic.

In one embodiment, the subject is further administered at least one additional compound useful for treating the breathing disorder or disease. In another embodiment, the at least one additional compound is selected from the group consisting of acetazolamide, almitrine, theophylline, caffeine, methylprogesterone and related compounds, a serotinergic modulator, a cannabinoid, and an ampakine. In yet another embodiment, the formulation is administered to the subject in conjunction with the use of a mechanical ventilation device or positive airway pressure device. In one embodiment, the formulation is administered to the subject by an inhalational, topical, oral, buccal, rectal, vaginal, intramuscular, subcutaneous, transdermal, intrathecal or intravenous route. In another embodiment, the subject is a mammal including but not limited to mouse, rat, ferret, guinea pig, monkey, dog, cat, horse, cow, pig and other farm animals.

In one embodiment, the subject is a human. In another embodiment, the at least one compound of formula (I) is selected from the group consisting of: N-(4,6-Bis-methylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(4,6-Bis-ethylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(4-Cyclopropylmethyl)-N-(6-n-propylamino)[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(4-Ethylamino)-N-(6-n-propylamino)-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(Bis-4,6-(2-methyl-propylamino)) [1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(Bis-4,6-(2,2-dimethylpropylamino)) [1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(Bis-4,6-(2,2-dimethylpropylamino))[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(4-(Methoxy(methyl)amino)-6-(n-propylamino)-1,3,5-triazin-2-yl)propionamide, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-methyl-hydroxylamine, O-Allyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-hydroxylamine, 6-(Methoxy(methyl)amino)-N^2-propyl-1,3,5-triazine-2,4-diamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N-methyl-hydroxylamine, O-Benzyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N-methyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N-isopropyl-hydroxylamine, 6-[1,2]Oxazinan-2-yl-N,N'-dipropyl-[1,3,5]triazine-2,4-diamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isopropyl-N-methyl-hydroxylamine, O-Benzyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N-ethyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isopropyl-hydroxylamine, 6-((Benzyloxy)(isopropyl)amino)-N^2,N^4-dipropyl-1,3,5-triazine-2,4-diamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N-ethyl-O-isopropyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isobutyl-N-methyl-hydroxylamine, 6-(Methyl(thiophen-2-ylmethoxy)amino)-N^2,N^4-dipropyl-1,3,5-triazine-2,4-diamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-cyclopropylmethyl-N-methyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-ethyl-N-methyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-(2,2-difluoro-ethyl)-hydroxylamine, 4-N-(2-Dimethylaminoethyl)amino-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, 4-N-(3-(1-N-Methylimidazol-2-yl)-propyl)-amino-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, 4-N-(1-N-Methylimidazol-2-yl)-methylamino-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, 4,6-Bis-(N-(2-dimethylaminoethyl)amino)-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, 4,6-Bis-(N-(pyridin-4-yl-methyl)amino)-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, 4,6-Bis-[N-(3-methoxy-n-propyl)amino]-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, 4,6-Bis-[N-(tetrahydropyran-4-yl-methyl)amino]-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, N-(5,8,11-Trioxa-2,14,16,18,19-pentaazabicyclo[13.3.1]nonadeca-1(18),15(19),16(17)-trien-17-yl)-N,O-dimethylhydroxylamine, 2,6-Bis-(N-n-propylamino)-[1,3]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N',N'-dimethylhydrazine, N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N-methyl-N'-methylhydrazine, 2-(n-Propyl)amino-4-(i-propylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine, 2-(n-Propyl)amino-4-dimethylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine, 2-(n-Propyl)amino-4-methylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine, 2-(n-Propyl)amino-4-(i-propyl)amino-7-i-propyl-pyrrolidino[2,3-d]pyrimidine, 2,4-Bis-(n-propyl)amino-7H-pyrrolidino[2,3-d]pyrimidine, 2-(n-Propyl)amino-4-(4-hydroxypiperidin-1-yl)-7-methyl-pyrrolidino[2,3-d]pyrimidine, 8-(7-Methyl-2-(n-propylamino)-pyrrolidino[2,3-d]pyrimidin-4-yl)-8-azabicyclo[3.2.1]octan-3-ol, N-(2-Propylamino-7H-pyrrolo[2,3d]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine, N-(2-(Propen-2-yl)amino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine, N-(2-(Propen-2-yl)amino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-O-methyl-hydroxylamine, N-(2-n-Propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine, N-(2-n-Propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-O-methyl-hydroxylamine, N-(2-n-Propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine, N-Methyl-N-(2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine, N,N-Dimethyl-N'-(2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine, N-(2,4-Bis-n-propylamino)-N-(6-methylamino)-[1,3,5]triazine, N-(2,4,6-Tris-n-propylamino)-[1,3,5]triazine, N-(2,4,6-Tris-n-propylamino)-1,3-pyrimidine, N-(2,4-Bis-n-propylamino)-N-(6-i-propylamino)-1,3-pyrimidine, a salt thereof and mixtures thereof.

Pharmaceutical Compositions and Formulations

The invention also encompasses the use of pharmaceutical compositions of at least one compound of the invention or a salt thereof to practice the methods of the invention.

Such a pharmaceutical composition may consist of at least one compound of the invention or a salt thereof, in a form suitable for administration to a subject, or the pharmaceutical composition may comprise at least one compound of the invention or a salt thereof, and one or more pharmaceutically acceptable carriers, one or more additional ingredients, or some combination of these. The at least one compound of the invention may be present in the pharmaceutical composition in the form of a physiologically acceptable salt, such as in combination with a physiologically acceptable cation or anion, as is well known in the art.

In an embodiment, the pharmaceutical compositions useful for practicing the method of the invention may be administered to deliver a dose of between 1 ng/kg/day and 100 mg/kg/day. In another embodiment, the pharmaceutical compositions useful for practicing the invention may be administered to deliver a dose of between 1 ng/kg/day and 500 mg/kg/day.

The relative amounts of the active ingredient, the pharmaceutically acceptable carrier, and any additional ingredients in a pharmaceutical composition of the invention will vary,

depending upon the identity, size, and condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

Pharmaceutical compositions that are useful in the methods of the invention may be suitably developed for inhalational, oral, rectal, vaginal, parenteral, topical, transdermal, pulmonary, intranasal, buccal, ophthalmic, intrathecal, intravenous or another route of administration. A composition useful within the methods of the invention may be directly administered to the brain, the brainstem, or any other part of the central nervous system of a mammal. Other contemplated formulations include projected nanoparticles, liposomal preparations, resealed erythrocytes containing the active ingredient, and immunologically-based formulations. The route(s) of administration will be readily apparent to the skilled artisan and will depend upon any number of factors including the type and severity of the disease being treated, the type and age of the veterinary or human patient being treated, and the like.

The formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a carrier or one or more other accessory ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single- or multi-dose unit.

As used herein, a "unit dose" is a discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient that would be administered to a subject or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage. The unit dosage form may be for a single daily dose or one of multiple daily doses (e.g., about 1 to 4 or more times per day). When multiple daily doses are used, the unit dosage form may be the same or different for each dose.

Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for ethical administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to animals of all sorts. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions of the invention is contemplated include, but are not limited to, humans and other primates, mammals including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, and dogs.

In one embodiment, the compositions of the invention are formulated using one or more pharmaceutically acceptable excipients or carriers. In one embodiment, the pharmaceutical compositions of the invention comprise a therapeutically effective amount of at least one compound of the invention and a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers, which are useful, include, but are not limited to, glycerol, water, saline, ethanol and other pharmaceutically acceptable salt solutions such as phosphates and salts of organic acids. Examples of these and other pharmaceutically acceptable carriers are described in Remington's Pharmaceutical Sciences (1991, Mack Publication Co., New Jersey).

The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity may be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms may be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, sodium chloride, or polyalcohols such as mannitol and sorbitol, in the composition. Prolonged absorption of the injectable compositions may be brought about by including in the composition an agent that delays absorption, for example, aluminum monostearate or gelatin. In one embodiment, the pharmaceutically acceptable carrier is not DMSO alone.

Formulations may be employed in admixtures with conventional excipients, i.e., pharmaceutically acceptable organic or inorganic carrier substances suitable for oral, parenteral, nasal, inhalational, intravenous, subcutaneous, transdermal enteral, or any other suitable mode of administration, known to the art. The pharmaceutical preparations may be sterilized and if desired mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure buffers, coloring, flavoring and/or aromatic substances and the like. They may also be combined where desired with other active agents, e.g., other analgesic agents. As used herein, "additional ingredients" include, but are not limited to, one or more ingredients that may be used as a pharmaceutical carrier.

The composition of the invention may comprise a preservative from about 0.005% to 2.0% by total weight of the composition. The preservative is used to prevent spoilage in the case of exposure to contaminants in the environment. Examples of preservatives useful in accordance with the invention included but are not limited to those selected from the group consisting of benzyl alcohol, sorbic acid, parabens, imidurea and combinations thereof. A particularly preferred preservative is a combination of about 0.5% to 2.0% benzyl alcohol and 0.05% to 0.5% sorbic acid.

The composition preferably includes an antioxidant and a chelating agent which inhibit the degradation of the compound. Preferred antioxidants for some compounds are BHT, BHA, alpha-tocopherol and ascorbic acid in the preferred range of about 0.01% to 0.3% and more preferably BHT in the range of 0.03% to 0.1% by weight by total weight of the composition. Preferably, the chelating agent is present in an amount of from 0.01% to 0.5% by weight by total weight of the composition. Particularly preferred chelating agents include edetate salts (e.g. disodium edetate) and citric acid in the weight range of about 0.01% to 0.20% and more preferably in the range of 0.02% to 0.10% by weight by total weight of the composition. The chelating agent is useful for chelating metal ions in the composition which may be detrimental to the shelf life of the formulation. While BHT and disodium edetate are the particularly preferred antioxidant and chelating agent respectively for some compounds, other suitable and equivalent antioxidants and chelating agents may be substituted therefore as would be known to those skilled in the art.

Liquid suspensions may be prepared using conventional methods to achieve suspension of the active ingredient in an aqueous or oily vehicle. Aqueous vehicles include, for

example, water, and isotonic saline. Oily vehicles include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin. Liquid suspensions may further comprise one or more additional ingredients including, but not limited to, suspending agents, dispersing or wetting agents, emulsifying agents, demulcents, preservatives, buffers, salts, flavorings, coloring agents, and sweetening agents. Oily suspensions may further comprise a thickening agent. Known suspending agents include, but are not limited to, sorbitol syrup, hydrogenated edible fats, sodium alginate, polyvinylpyrrolidone, gum tragacanth, gum acacia, and cellulose derivatives such as sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose. Known dispersing or wetting agents include, but are not limited to, naturally-occurring phosphatides such as lecithin, condensation products of an alkylene oxide with a fatty acid, with a long chain aliphatic alcohol, with a partial ester derived from a fatty acid and a hexitol, or with a partial ester derived from a fatty acid and a hexitol anhydride (e.g., polyoxyethylene stearate, heptadecaethyleneoxycetanol, polyoxyethylene sorbitol monooleate, and polyoxyethylene sorbitan monooleate, respectively). Known emulsifying agents include, but are not limited to, lecithin, and acacia. Known preservatives include, but are not limited to, methyl, ethyl, or n-propyl para-hydroxybenzoates, ascorbic acid, and sorbic acid. Known sweetening agents include, for example, glycerol, propylene glycol, sorbitol, sucrose, and saccharin. Known thickening agents for oily suspensions include, for example, beeswax, hard paraffin, and cetyl alcohol.

Liquid solutions of the active ingredient in aqueous or oily solvents may be prepared in substantially the same manner as liquid suspensions, the primary difference being that the active ingredient is dissolved, rather than suspended in the solvent. As used herein, an "oily" liquid is one which comprises a carbon-containing liquid molecule and which exhibits a less polar character than water. Liquid solutions of the pharmaceutical composition of the invention may comprise each of the components described with regard to liquid suspensions, it being understood that suspending agents will not necessarily aid dissolution of the active ingredient in the solvent. Aqueous solvents include, for example, water, and isotonic saline. Oily solvents include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin.

Powdered and granular formulations of a pharmaceutical preparation of the invention may be prepared using known methods. Such formulations may be administered directly to a subject, used, for example, to form tablets, to fill capsules, or to prepare an aqueous or oily suspension or solution by addition of an aqueous or oily vehicle thereto. Each of these formulations may further comprise one or more of dispersing or wetting agent, a suspending agent, and a preservative. Additional excipients, such as fillers and sweetening, flavoring, or coloring agents, may also be included in these formulations.

A pharmaceutical composition of the invention may also be prepared, packaged, or sold in the form of oil-in-water emulsion or a water-in-oil emulsion. The oily phase may be a vegetable oil such as olive or arachis oil, a mineral oil such as liquid paraffin, or a combination of these. Such compositions may further comprise one or more emulsifying agents such as naturally occurring gums such as gum acacia or gum tragacanth, naturally-occurring phosphatides such as soybean or lecithin phosphatide, esters or partial esters derived from combinations of fatty acids and hexitol anhydrides such as sorbitan monooleate, and condensation products of such partial esters with ethylene oxide such as polyoxyethylene sorbitan monooleate. These emulsions may also contain additional ingredients including, for example, sweetening or flavoring agents.

Methods for impregnating or coating a material with a chemical composition are known in the art, and include, but are not limited to methods of depositing or binding a chemical composition onto a surface, methods of incorporating a chemical composition into the structure of a material during the synthesis of the material (i.e., such as with a physiologically degradable material), and methods of absorbing an aqueous or oily solution or suspension into an absorbent material, with or without subsequent drying.

Administration/Dosing

The regimen of administration may affect what constitutes an effective amount. The therapeutic formulations may be administered to the patient either prior to or after the onset of a breathing disorder event. Further, several divided dosages, as well as staggered dosages may be administered daily or sequentially, or the dose may be continuously infused, or may be a bolus injection. Further, the dosages of the therapeutic formulations may be proportionally increased or decreased as indicated by the exigencies of the therapeutic or prophylactic situation.

Administration of the compositions of the present invention to a patient, preferably a mammal or avian, more preferably a human, may be carried out using known procedures, at dosages and for periods of time effective to treat a breathing control disorder in the patient. An effective amount of the therapeutic compound necessary to achieve a therapeutic effect may vary according to factors such as the activity of the particular compound employed; the time of administration; the rate of excretion of the compound; the duration of the treatment; other drugs, compounds or materials used in combination with the compound; the state of the disease or disorder, age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well-known in the medical arts. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. A non-limiting example of an effective dose range for a therapeutic compound of the invention is from about 0.01 mg/kg and 50 mg/kg of body weight/per day. One of ordinary skill in the art would be able to study the relevant factors and make the determination regarding the effective amount of the therapeutic compound without undue experimentation.

The compound can be administered to an animal as frequently as several times daily, or it may be administered less frequently, such as once a day, once a week, once every two weeks, once a month, or even less frequently, such as once every several months or even once a year or less. It is understood that the amount of compound dosed per day may be administered, in non-limiting examples, every day, every other day, every 2 days, every 3 days, every 4 days, or every 5 days. For example, with every other day administration, a 5 mg per day dose may be initiated on Monday with a first subsequent 5 mg per day dose administered on Wednesday, a second subsequent 5 mg per day dose administered on Friday, and so on. The frequency of the dose will be readily apparent to the skilled artisan and will depend upon any number of factors, such as, but not limited to, the type and severity of the disease being treated, the type and age of the animal, etc.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

A medical doctor, e.g., physician or veterinarian, having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

In particular embodiments, it is especially advantageous to formulate the compound in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the patients to be treated; each unit containing a predetermined quantity of therapeutic compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical vehicle. The dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the therapeutic compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding/formulating such a therapeutic compound for the treatment of breathing disorders in a patient.

In one embodiment, the compositions of the invention are administered to the patient in dosages that range from one to five times per day or more. In another embodiment, the compositions of the invention are administered to the patient in range of dosages that include, but are not limited to, once every day, every two, days, every three days to once a week, and once every two weeks. It will be readily apparent to one skilled in the art that the frequency of administration of the various combination compositions of the invention will vary from subject to subject depending on many factors including, but not limited to, age, disease or disorder to be treated, gender, overall health, and other factors. Thus, the invention should not be construed to be limited to any particular dosage regime and the precise dosage and composition to be administered to any patient will be determined by the attending physical taking all other factors about the patient into account.

Compounds of the invention for administration may be in the range of from about 1 μg to about 7,500 mg, about 20 μg to about 7,000 mg, about 40 μg to about 6,500 mg, about 80 μg to about 6,000 mg, about 100 μg to about 5,500 mg, about 200 μg to about 5,000 mg, about 400 μg to about 4,000 mg, about 800 μg to about 3,000 mg, about 1 mg to about 2,500 mg, about 2 mg to about 2,000 mg, about 5 mg to about 1,000 mg, about 10 mg to about 750 mg, about 20 mg to about 600 mg, about 30 mg to about 500 mg, about 40 mg to about 400 mg, about 50 mg to about 300 mg, about 60 mg to about 250 mg, about 70 mg to about 200 mg, about 80 mg to about 150 mg, and any and all whole or partial increments thereinbetween.

In some embodiments, the dose of a compound of the invention is from about 0.5 μg and about 5,000 mg. In some embodiments, a dose of a compound of the invention used in compositions described herein is less than about 5,000 mg, or less than about 4,000 mg, or less than about 3,000 mg, or less than about 2,000 mg, or less than about 1,000 mg, or less than about 800 mg, or less than about 600 mg, or less than about 500 mg, or less than about 200 mg, or less than about 50 mg. Similarly, in some embodiments, a dose of a second compound as described herein is less than about 1,000 mg, or less than about 800 mg, or less than about 600 mg, or less than about 500 mg, or less than about 400 mg, or less than about 300 mg, or less than about 200 mg, or less than about 100 mg, or less than about 50 mg, or less than about 40 mg, or less than about 30 mg, or less than about 25 mg, or less than about 20 mg, or less than about 15 mg, or less than about 10 mg, or less than about 5 mg, or less than about 2 mg, or less than about 1 mg, or less than about 0.5 mg, and any and all whole or partial increments thereof.

In one embodiment, the present invention is directed to a packaged pharmaceutical composition comprising a container holding a therapeutically effective amount of a compound of the invention, alone or in combination with a second pharmaceutical agent; and instructions for using the compound to treat, prevent, or reduce one or more symptoms of breathing disorder in a patient.

The term "container" includes any receptacle for holding the pharmaceutical composition. For example, in one embodiment, the container is the packaging that contains the pharmaceutical composition. In other embodiments, the container is not the packaging that contains the pharmaceutical composition, i.e., the container is a receptacle, such as a box or vial that contains the packaged pharmaceutical composition or unpackaged pharmaceutical composition and the instructions for use of the pharmaceutical composition. Moreover, packaging techniques are well known in the art. It should be understood that the instructions for use of the pharmaceutical composition may be contained on the packaging containing the pharmaceutical composition, and as such the instructions form an increased functional relationship to the packaged product. However, it should be understood that the instructions may contain information pertaining to the compound's ability to perform its intended function, e.g., treating, preventing, or reducing a breathing disorder in a patient.

Routes of Administration

Routes of administration of any of the compositions of the invention include inhalational, oral, nasal, rectal, parenteral, sublingual, transdermal, transmucosal (e.g., sublingual, lingual, (trans)buccal, (trans)urethral, vaginal (e.g., trans- and perivaginally), (intra)nasal, and (trans)rectal), intravesical, intrapulmonary, intraduodenal, intragastrical, intrathecal, subcutaneous, intramuscular, intradermal, intra-arterial, intravenous, intrabronchial, inhalation, intraperitoneal, intrathoracic, intrapleural and topical administration.

Suitable compositions and dosage forms include, for example, tablets, capsules, caplets, pills, gel caps, troches, dispersions, suspensions, solutions, syrups, granules, beads, transdermal patches, gels, powders, pellets, magmas, lozenges, creams, pastes, plasters, lotions, discs, suppositories, liquid sprays for nasal or oral administration, dry powder or aerosolized formulations for inhalation, compositions and formulations for intravesical administration and the like. It should be understood that the formulations and compositions that would be useful in the present invention are not limited to the particular formulations and compositions that are described herein.

Oral Administration

For oral application, particularly suitable are tablets, dragees, liquids, drops, suppositories, or capsules, caplets and gelcaps. Other formulations suitable for oral administration include, but are not limited to, a powdered or granular formulation, an aqueous or oily suspension, an aqueous or oily solution, a paste, a gel, toothpaste, a mouthwash, a coating, an oral rinse, or an emulsion. The compositions intended for oral use may be prepared according to any method known in the art and such compositions may contain one or more agents

selected from the group consisting of inert, non-toxic pharmaceutically excipients which are suitable for the manufacture of tablets. Such excipients include, for example an inert diluent such as lactose; granulating and disintegrating agents such as cornstarch; binding agents such as starch; and lubricating agents such as magnesium stearate.

Tablets may be non-coated or they may be coated using known methods to achieve delayed disintegration in the gastrointestinal tract of a subject, thereby providing sustained release and absorption of the active ingredient. By way of example, a material such as glyceryl monostearate or glyceryl distearate may be used to coat tablets. Further by way of example, tablets may be coated using methods described in U.S. Pat. Nos. 4,256,108; 4,160,452; and 4,265,874 to form osmotically controlled release tablets. Tablets may further comprise a sweetening agent, a flavoring agent, a coloring agent, a preservative, or some combination of these in order to provide for pharmaceutically elegant and palatable preparation.

Hard capsules comprising the active ingredient may be made using a physiologically degradable composition, such as gelatin. Such hard capsules comprise the active ingredient, and may further comprise additional ingredients including, for example, an inert solid diluent such as calcium carbonate, calcium phosphate, or kaolin.

Soft gelatin capsules comprising the active ingredient may be made using a physiologically degradable composition, such as gelatin. Such soft capsules comprise the active ingredient, which may be mixed with water or an oil medium such as peanut oil, liquid paraffin, or olive oil.

For oral administration, the compounds of the invention may be in the form of tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents; fillers; lubricants; disintegrates; or wetting agents. If desired, the tablets may be coated using suitable methods and coating materials such as OPADRY™ film coating systems available from Colorcon, West Point, Pa. (e.g., OPADRY™ OY Type, OYC Type, Organic Enteric OY-P Type, Aqueous Enteric OY-A Type, OY-PM Type and OPADRY™ White, 32K18400).

Liquid preparation for oral administration may be in the form of solutions, syrups or suspensions. The liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, methyl cellulose or hydrogenated edible fats); emulsifying agent (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters or ethyl alcohol); and preservatives (e.g., methyl or propyl para-hydroxy benzoates or sorbic acid). Liquid formulations of a pharmaceutical composition of the invention which are suitable for oral administration may be prepared, packaged, and sold either in liquid form or in the form of a dry product intended for reconstitution with water or another suitable vehicle prior to use.

A tablet comprising the active ingredient may, for example, be made by compressing or molding the active ingredient, optionally with one or more additional ingredients. Compressed tablets may be prepared by compressing, in a suitable device, the active ingredient in a free-flowing form such as a powder or granular preparation, optionally mixed with one or more of a binder, a lubricant, an excipient, a surface active agent, and a dispersing agent. Molded tablets may be made by molding, in a suitable device, a mixture of the active ingredient, a pharmaceutically acceptable carrier, and at least sufficient liquid to moisten the mixture. Pharmaceutically acceptable excipients used in the manufacture of tablets include, but are not limited to, inert diluents, granulating and disintegrating agents, binding agents, and lubricating agents. Known dispersing agents include, but are not limited to, potato starch and sodium starch glycollate. Known surface-active agents include, but are not limited to, sodium lauryl sulphate. Known diluents include, but are not limited to, calcium carbonate, sodium carbonate, lactose, microcrystalline cellulose, calcium phosphate, calcium hydrogen phosphate, and sodium phosphate. Known granulating and disintegrating agents include, but are not limited to, corn starch and alginic acid. Known binding agents include, but are not limited to, gelatin, acacia, pre-gelatinized maize starch, polyvinylpyrrolidone, and hydroxypropyl methylcellulose. Known lubricating agents include, but are not limited to, magnesium stearate, stearic acid, silica, and talc.

Granulating techniques are well known in the pharmaceutical art for modifying starting powders or other particulate materials of an active ingredient. The powders are typically mixed with a binder material into larger permanent free-flowing agglomerates or granules referred to as a "granulation." For example, solvent-using "wet" granulation processes are generally characterized in that the powders are combined with a binder material and moistened with water or an organic solvent under conditions resulting in the formation of a wet granulated mass from which the solvent must then be evaporated.

Melt granulation generally consists in the use of materials that are solid or semi-solid at room temperature (i.e. having a relatively low softening or melting point range) to promote granulation of powdered or other materials, essentially in the absence of added water or other liquid solvents. The low melting solids, when heated to a temperature in the melting point range, liquefy to act as a binder or granulating medium. The liquefied solid spreads itself over the surface of powdered materials with which it is contacted, and on cooling, forms a solid granulated mass in which the initial materials are bound together. The resulting melt granulation may then be provided to a tablet press or be encapsulated for preparing the oral dosage form. Melt granulation improves the dissolution rate and bioavailability of an active (i.e. drug) by forming a solid dispersion or solid solution.

U.S. Pat. No. 5,169,645 discloses directly compressible wax-containing granules having improved flow properties. The granules are obtained when waxes are admixed in the melt with certain flow improving additives, followed by cooling and granulation of the admixture. In certain embodiments, only the wax itself melts in the melt combination of the wax(es) and additives(s), and in other cases both the wax(es) and the additives(s) will melt.

The present invention also includes a multi-layer tablet comprising a layer providing for the delayed release of one or more compounds useful within the methods of the invention, and a further layer providing for the immediate release of one or more compounds useful within the methods of the invention. Using a wax/pH-sensitive polymer mix, a gastric insoluble composition may be obtained in which the active ingredient is entrapped, ensuring its delayed release.

Parenteral Administration

As used herein, "parenteral administration" of a pharmaceutical composition includes any route of administration characterized by physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue. Parenteral administration thus includes, but is not limited to, administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-penetrating non-surgical wound, and the like. In particular, parenteral

administration is contemplated to include, but is not limited to, subcutaneous, intravenous, intraperitoneal, intramuscular, intrasternal injection, and kidney dialytic infusion techniques.

Formulations of a pharmaceutical composition suitable for parenteral administration comprise the active ingredient combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampules or in multi-dose containers containing a preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations. Such formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a formulation for parenteral administration, the active ingredient is provided in dry (i.e., powder or granular) form for reconstitution with a suitable vehicle (e.g., sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition.

The pharmaceutical compositions may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulations may be prepared using a non-toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butane diol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or di-glycerides. Other parentally-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer system. Compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.

Topical Administration

An obstacle for topical administration of pharmaceuticals is the stratum corneum layer of the epidermis. The stratum corneum is a highly resistant layer comprised of protein, cholesterol, sphingolipids, free fatty acids and various other lipids, and includes cornified and living cells. One of the factors that limit the penetration rate (flux) of a compound through the stratum corneum is the amount of the active substance that can be loaded or applied onto the skin surface. The greater the amount of active substance which is applied per unit of area of the skin, the greater the concentration gradient between the skin surface and the lower layers of the skin, and in turn the greater the diffusion force of the active substance through the skin. Therefore, a formulation containing a greater concentration of the active substance is more likely to result in penetration of the active substance through the skin, and more of it, and at a more consistent rate, than a formulation having a lesser concentration, all other things being equal.

Formulations suitable for topical administration include, but are not limited to, liquid or semi-liquid preparations such as liniments, lotions, oil-in-water or water-in-oil emulsions such as creams, ointments or pastes, and solutions or suspensions. Topically administrable formulations may, for example, comprise from about 1% to about 10% (w/w) active ingredient, although the concentration of the active ingredient may be as high as the solubility limit of the active ingredient in the solvent. Formulations for topical administration may further comprise one or more of the additional ingredients described herein.

Enhancers of permeation may be used. These materials increase the rate of penetration of drugs across the skin. Typical enhancers in the art include ethanol, glycerol monolaurate, PGML (polyethylene glycol monolaurate), dimethylsulfoxide, and the like. Other enhancers include oleic acid, oleyl alcohol, ethoxydiglycol, laurocapram, alkanecarboxylic acids, dimethylsulfoxide, polar lipids, or N-methyl-2-pyrrolidone.

One acceptable vehicle for topical delivery of some of the compositions of the invention may contain liposomes. The composition of the liposomes and their use are known in the art (for example, see Constanza, U.S. Pat. No. 6,323,219).

In alternative embodiments, the topically active pharmaceutical composition may be optionally combined with other ingredients such as adjuvants, antioxidants, chelating agents, surfactants, foaming agents, wetting agents, emulsifying agents, viscosifiers, buffering agents, preservatives, and the like. In another embodiment, a permeation or penetration enhancer is included in the composition and is effective in improving the percutaneous penetration of the active ingredient into and through the stratum corneum with respect to a composition lacking the permeation enhancer. Various permeation enhancers, including oleic acid, oleyl alcohol, ethoxydiglycol, laurocapram, alkanecarboxylic acids, dimethylsulfoxide, polar lipids, or N-methyl-2-pyrrolidone, are known to those of skill in the art. In another aspect, the composition may further comprise a hydrotropic agent, which functions to increase disorder in the structure of the stratum corneum, and thus allows increased transport across the stratum corneum. Various hydrotropic agents such as isopropyl alcohol, propylene glycol, or sodium xylene sulfonate, are known to those of skill in the art.

The topically active pharmaceutical composition should be applied in an amount effective to affect desired changes. As used herein "amount effective" shall mean an amount sufficient to cover the region of skin surface where a change is desired. An active compound should be present in the amount of from about 0.0001% to about 15% by weight volume of the composition. More preferable, it should be present in an amount from about 0.0005% to about 5% of the composition; most preferably, it should be present in an amount of from about 0.001% to about 1% of the composition. Such compounds may be synthetically-or naturally derived.

Buccal Administration

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for buccal administration. Such formulations may, for example, be in the form of tablets or lozenges made using conventional methods, and may contain, for example, 0.1 to 20% (w/w) of the active ingredient, the balance comprising an orally dissolvable or degradable composition and, optionally, one or more of the additional ingredients described herein. Alternately, formulations suitable for buccal administration may comprise a powder or an aerosolized or atomized solution or suspension comprising the active ingredient. Such powdered, aerosolized, or aerosolized formulations, when dispersed, preferably have an average particle or droplet size in the range from about 0.1 to about 200 nanometers, and may further comprise one or more of the additional ingredients described herein. The examples of formulations described herein are

not exhaustive and it is understood that the invention includes additional modifications of these and other formulations not described herein, but which are known to those of skill in the art.

Rectal Administration

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for rectal administration. Such a composition may be in the form of, for example, a suppository, a retention enema preparation, and a solution for rectal or colonic irrigation.

Suppository formulations may be made by combining the active ingredient with a non-irritating pharmaceutically acceptable excipient which is solid at ordinary room temperature (i.e., about 20° C.) and which is liquid at the rectal temperature of the subject (i.e., about 37° C. in a healthy human). Suitable pharmaceutically acceptable excipients include, but are not limited to, cocoa butter, polyethylene glycols, and various glycerides. Suppository formulations may further comprise various additional ingredients including, but not limited to, antioxidants, and preservatives.

Retention enema preparations or solutions for rectal or colonic irrigation may be made by combining the active ingredient with a pharmaceutically acceptable liquid carrier. As is well known in the art, enema preparations may be administered using, and may be packaged within, a delivery device adapted to the rectal anatomy of the subject. Enema preparations may further comprise various additional ingredients including, but not limited to, antioxidants, and preservatives.

Additional Administration Forms

Additional dosage forms of this invention include dosage forms as described in U.S. Pat. Nos. 6,340,475, 6,488,962, 6,451,808, 5,972,389, 5,582,837, and 5,007,790. Additional dosage forms of this invention also include dosage forms as described in U.S. Patent Applications Nos. 20030147952, 20030104062, 20030104053, 20030044466, 20030039688, and 20020051820. Additional dosage forms of this invention also include dosage forms as described in PCT Applications Nos. WO 03/35041, WO 03/35040, WO 03/35029, WO 03/35177, WO 03/35039, WO 02/96404, WO 02/32416, WO 01/97783, WO 01/56544, WO 01/32217, WO 98/55107, WO 98/11879, WO 97/47285, WO 93/18755, and WO 90/11757.

Controlled Release Formulations and Drug Delivery Systems

Controlled- or sustained-release formulations of a pharmaceutical composition of the invention may be made using conventional technology. In some cases, the dosage forms to be used can be provided as slow or controlled-release of one or more active ingredients therein using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, or microspheres or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled-release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the pharmaceutical compositions of the invention. Thus, single unit dosage forms suitable for oral administration, such as tablets, capsules, gelcaps, and caplets, that are adapted for controlled-release are encompassed by the present invention.

Most controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled counterparts. Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include extended activity of the drug, reduced dosage frequency, and increased patient compliance. In addition, controlled-release formulations can be used to affect the time of onset of action or other characteristics, such as blood level of the drug, and thus can affect the occurrence of side effects.

Most controlled-release formulations are designed to initially release an amount of drug that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body.

Controlled-release of an active ingredient can be stimulated by various inducers, for example pH, temperature, enzymes, water, or other physiological conditions or compounds. The term "controlled-release component" in the context of the present invention is defined herein as a compound or compounds, including, but not limited to, polymers, polymer matrices, gels, permeable membranes, liposomes, or microspheres or a combination thereof that facilitates the controlled-release of the active ingredient.

In certain embodiments, the formulations of the present invention may be, but are not limited to, short-term, rapid-offset, as well as controlled, for example, sustained release, delayed release and pulsatile release formulations.

The term sustained release is used in its conventional sense to refer to a drug formulation that provides for gradual release of a drug over an extended period of time, and that may, although not necessarily, result in substantially constant blood levels of a drug over an extended time period. The period of time may be as long as a month or more and should be a release that is longer that the same amount of agent administered in bolus form.

For sustained release, the compounds may be formulated with a suitable polymer or hydrophobic material which provides sustained release properties to the compounds. As such, the compounds for use the method of the invention may be administered in the form of microparticles, for example, by injection or in the form of wafers or discs by implantation.

In a preferred embodiment of the invention, the compounds of the invention are administered to a patient, alone or in combination with another pharmaceutical agent, using a sustained release formulation.

The term delayed release is used herein in its conventional sense to refer to a drug formulation that provides for an initial release of the drug after some delay following drug administration and that may, although not necessarily, includes a delay of from about 10 minutes up to about 12 hours.

The term pulsatile release is used herein in its conventional sense to refer to a drug formulation that provides release of the drug in such a way as to produce pulsed plasma profiles of the drug after drug administration.

The term immediate release is used in its conventional sense to refer to a drug formulation that provides for release of the drug immediately after drug administration.

As used herein, short-term refers to any period of time up to and including about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4 hours, about 3 hours, about 2 hours, about 1 hour, about 40 minutes, about 20 minutes, or about 10 minutes and any or all whole or partial increments thereof after drug administration after drug administration.

As used herein, rapid-offset refers to any period of time up to and including about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4 hours, about 3 hours, about 2 hours, about 1 hour, about 40 minutes, about 20 minutes, or about 10 minutes, and any and all whole or partial increments thereof after drug administration.

Mechanical Devices

In one aspect of the invention, a method of treating a patient lacking normal breathing and normal breathing control comprises administering the composition useful within the invention as described herein, and additionally treating the patient using a device for treatment of a lack of normal breathing. Such devices include, but are not limited to, ventilation devices, CPAP and BiPAP devices.

Mechanical ventilation is a method to mechanically assist or replace spontaneous breathing. Mechanical ventilation is typically used after an invasive intubation, a procedure wherein an endotracheal or tracheostomy tube is inserted into the airway. It is normally used in acute settings, such as in the operating room or ICU, for a short period of time during surgery, medical procedure, or serious illness. It may also be used at home or in a nursing or rehabilitation institution, if patients have chronic illnesses that require long-term ventilation assistance. The main form of mechanical ventilation is positive pressure ventilation, which works by increasing the pressure in the patient's airway and thus forcing air into the lungs. Less common today are negative pressure ventilators (for example, the "iron lung") that create a negative pressure environment around the patient's chest, thus sucking air into the lungs. Mechanical ventilation is often a life-saving intervention, but carries many potential complications including pneumothorax, airway injury, alveolar damage, and ventilator-associated pneumonia. For this reason the pressure and volume of gas used is strictly controlled, and reduced as soon as possible. Types of mechanical ventilation are: conventional ventilation, high frequency ventilation, non-invasive ventilation (non-invasive positive pressure pentilation or NIPPV), proportional assist ventilation (PAV), adaptive support ventilation (ASV) and neurally adjusted ventilatory assist (NAVA).

Non-invasive ventilation refers to all modalities that assist ventilation without the use of an endotracheal tube. Non-invasive ventilation is primarily aimed at minimizing patient discomfort and the complications associated with invasive ventilation, and is often used in cardiac disease, exacerbations of chronic pulmonary disease, sleep apnea, and neuromuscular diseases. Non-invasive ventilation refers only to the patient interface and not the mode of ventilation used; modes may include spontaneous or control modes and may be either pressure or volume modes. Some commonly used modes of NIPPV include:

(a) Continuous positive airway pressure (CPAP): This kind of machine has been used mainly by patients for the treatment of sleep apnea at home, but now is in widespread use across intensive care units as a form of ventilation. The CPAP machine stops upper airway obstruction by delivering a stream of compressed air via a hose to a nasal pillow, nose mask or full-face mask, splinting the airway (keeping it open under air pressure) so that unobstructed breathing becomes possible, reducing and/or preventing apneas and hypopneas. When the machine is turned on, but prior to the mask being placed on the head, a flow of air comes through the mask. After the mask is placed on the head, it is sealed to the face and the air stops flowing. At this point, it is only the air pressure that accomplishes the desired result. This has the additional benefit of reducing or eliminating the extremely loud snoring that sometimes accompanies sleep apnea.

(b) Bi-level positive airway pressure (BIPAP): Pressures alternate between inspiratory positive airway pressure (IPAP) and a lower expiratory positive airway pressure (EPAP), triggered by patient effort. On many such devices, backup rates may be set, which deliver IPAP pressures even if patients fail to initiate a breath.

(c) Intermittent positive pressure ventilation (IPPV), via mouthpiece or mask.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures, embodiments, claims, and examples described herein. Such equivalents were considered to be within the scope of this invention and covered by the claims appended hereto. For example, it should be understood, that modifications in reaction conditions, including but not limited to reaction times, reaction size/volume, and experimental reagents, such as solvents, catalysts, pressures, atmospheric conditions, e.g., nitrogen atmosphere, and reducing/oxidizing agents, with art-recognized alternatives and using no more than routine experimentation, are within the scope of the present application.

It is to be understood that, wherever values and ranges are provided herein, the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, all values and ranges encompassed by these values and ranges are meant to be encompassed within the scope of the present invention. Moreover, all values that fall within these ranges, as well as the upper or lower limits of a range of values, are also contemplated by the present application. The description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range and, when appropriate, partial integers of the numerical values within ranges. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

The following examples further illustrate aspects of the present invention. However, they are in no way a limitation of the teachings or disclosure of the present invention as set forth herein.

EXAMPLES

The invention is now described with reference to the following Examples. These Examples are provided for the purpose of illustration only, and the invention is not limited to these Examples, but rather encompasses all variations that are evident as a result of the teachings provided herein.

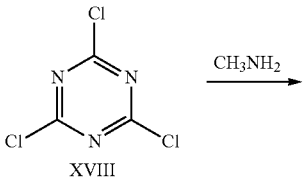
Materials:

Unless otherwise noted, all remaining starting materials were obtained from commercial suppliers and used without purification. Final products are typically isolated as hydrochloride acid addition salts unless noted otherwise.

Example 1

N-(4,6-Bis-methylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (XX)

Scheme 8



XVIII

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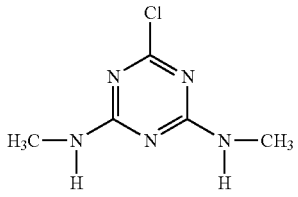
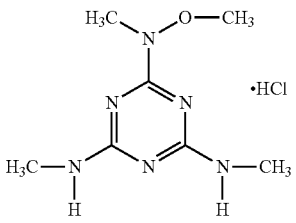


XIX



XX

2-Chloro-N-(4,6-bis-methylamino)-[1,3,5]triazine (XIX)

2,4,6-Trichloro-1,3,5-triazine (XVIII) (5.0 g, 27 mmol) was dissolved in acetone (35 mL) and poured into ice-water (50 mL) to form a very fine suspension. A solution of N-methylamine hydrochloride (3.66 g, 54 mmol) in water (20 mL) was added and the temperature maintained at approximately 0° C. To this mixture, 2N NaOH (54 mL, 108 mmol) was added in a dropwise manner to keep the temperature between 0° C. and 5° C. The mixture was stirred 30 min at ambient temperature for an additional 60 min at 50° C. The precipitate was filtered and washed with water (3×25 mL). After drying over anhydrous calcium chloride under high vacuum, 2-chloro-N-(4,6-bis-methylamino)-[1,3,5]triazine (XIX) was isolated as a white powder (4.2 g, 89% yield). LCMS (ESI) m/z=174 $(M+H)^+$.

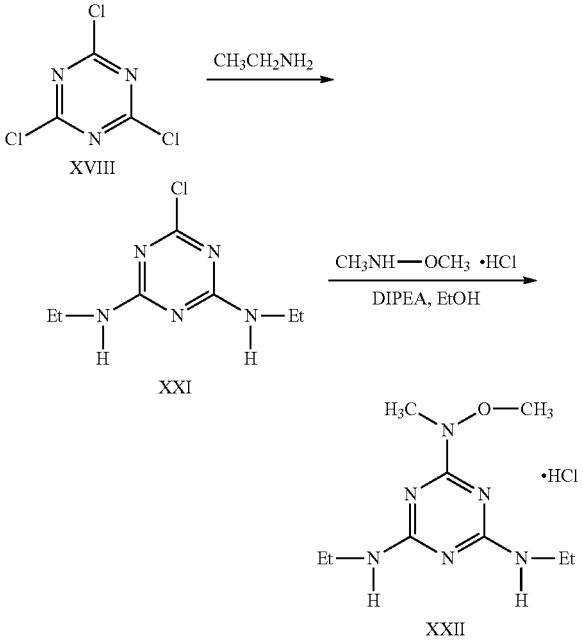
N-(4,6-Bis-methylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (XX)

A mixture of 2-chloro-N-(4,6-bis-methylamino)-[1,3,5] triazine (XIX) (1.74 g, 10 mmol), N,O-dimethylhydroxylamine hydrochloride (3.88 g, 40 mmol) and DIPEA (7.74 g, 60 mmol) in EtOH (200 mL) was heated at 100° C. for 16 h, after which the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (150 mL), washed with water (100 mL) and brine solution (100 mL), and then dried over Na_2SO_4. The solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (pet ether/ethyl acetate=5/1 to 5/3) to yield 899 mg (23%) of the desired product. The isolated free amine (380 mg, 2 mmol) was placed into H_2O (10 mL) and 0.5 M aqueous HCl solution (6 mL) was added. The resultant solution was subjected to lyophilization to yield the desired product, N-(4,6-bis-methylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (XX), as a white solid (468 mg). LCMS (ESI) m/z=199 $(M+H)^+$. ^1H NMR (500 MHz, DMSO): δ (ppm) 12.20-12.50 (br, 1H), 8.48-8.62 (m, 2H), 3.76-3.86 (m, 3H), 3.29-3.39 (m, 3H), 2.76-2.93 (m, 6H).

Example 2

N-(4,6-Bis-ethylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (XXII)

Scheme 9



XVIII

XXI

XXII

2-Chloro-N-(4,6-bis-ethylamino)-[1,3,5]triazine (XXI)

2,4,6-Trichloro-1,3,5-triazine (XVIII) (5.0 g, 27 mmol) was dissolved in acetone (35 mL) and poured into ice-water (50 mL) to form a very fine suspension. A solution of ethylamine (2.43 g, 54 mmol) in water (20 mL) was added and the temperature maintained at approximately 0° C. To this mixture, 2N NaOH (27 mL, 54 mmol) was added in a dropwise manner to keep the temperature between 0° C. and 5° C. The mixture was stirred for 30 min at ambient temperature, and for additional 60 min at 50° C. The precipitate was filtered off, washed with water (3×25 mL). After drying over anhydrous calcium chloride under high vacuum, 2-chloro-N-(4,6-bis-ethylamino)-[1,3,5]triazine (XXI) was isolated as a white powder (5.0 g, 92% yield). LCMS (ESI) m/z=202 $(M+H)^+$.

N-(4,6-Bis-ethylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (XXII)

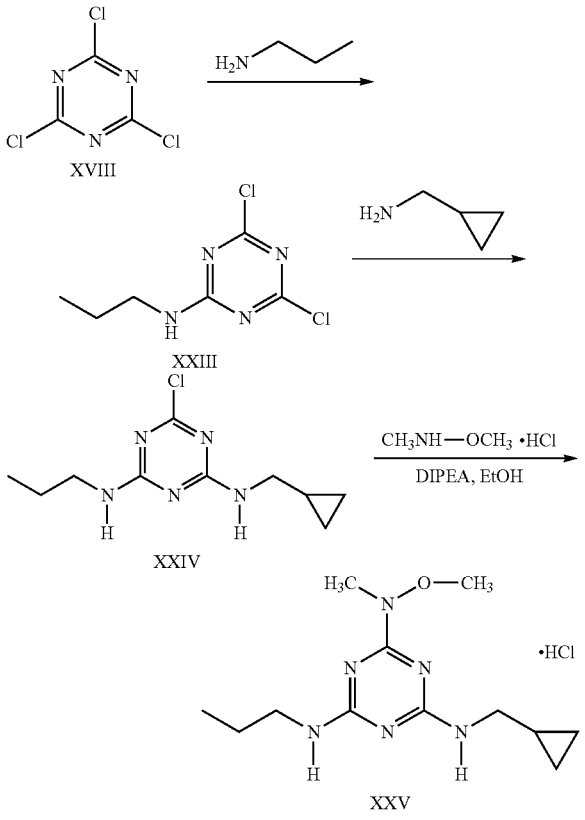
A mixture of 2-chloro-N-(4,6-bis-ethylamino)-[1,3,5]triazine (XXI) (4.03 g, 20 mmol), N,O-dimethylhydroxylamine hydrochloride (9.7 g, 100 mmol) and DIPEA (1.806 g, 140 mmol) in EtOH (200 mL) was heated at 100° C. for 16 h. After this time, the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (400 mL), washed with water (100 mL) and a brine solution (100 mL), then dried over Na_2SO_4 and concentrated. The crude product was purified by flash column chromatography (pet ether/ethyl acetate=5/1 to

5/2) to yield 811 mg (18%) of the desired product. The isolated free amine (811 mg, 3.58 mmol) was dissolved in H_2O (10 mL) and 0.5 M HCl solution in H_2O (7.2 mL) was added. The resultant solution was lyophilized to yield the desired product, N-(4,6-bis-ethylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (XXII) as a white solid (938 mg). LCMS (ESI) m/z=227 $(M+H)^+$. ^1H NMR (500 MHz, DMSO): δ (ppm) 12.40-12.80 (br, 1H), 8.58-8.87 (m, 2H), 3.76-3.78 (m, 4H), 3.34-3.37 (m, 6H), 1.10-1.16 (m, 6H).

Example 3

N-(4-Cyclopropylmethyl)-N-(6-n-propylamino) [1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXV)

Scheme 10



2,4-Dichloro-N-(6-n-propylamino)-[1,3,5]triazine (XXIII)

2,4,6-Trichloro-1,3,5-triazine (XVIII) (20 g, 109 mmol) was dissolved in acetone (100 mL) and poured into ice-water (50 mL) to form a very fine suspension. A solution of propan-1-amine (7.1 g, 120 mmol) in water (20 mL) was added and the temperature maintained at approximately 0° C. To this mixture, 2N NaOH (60 mL, 120 mmol) was added in a dropwise manner to keep the temperature between −5° C. and 0° C. The mixture was stirred at 0° C. for 60 min. The precipitate was filtered off and washed with water (3×25 mL). After drying over calcium chloride under high vacuum, 2,4-dichloro-N-(6-n-propylamino)-[1,3,5]triazine (XVIII) was isolated as a white powder (18 g, 80% yield). LCMS (ESI) m/z=208 $(M+H)^+$.

2-Chloro-N-(4-cyclopropylmethyl)-N-(6-n-propylamino) [1,3,5]triazine (XXIV)

2,4-Dichloro-N-(6-n-propylamino)-[1,3,5]triazine (XVIII) (18 g, 87 mmol) was dissolved in acetone (100 mL) and poured into ice-water (50 mL) to form a very fine suspension. A solution of cyclopropylmethanamine (6.7 g, 95 mmol) in acetone (30 mL) was added and the temperature was maintained at approximately 0° C. To this mixture, 2N NaOH (44 mL, 88 mmol) was added in a dropwise manner to keep the temperature between 0° C. and 5° C. The mixture was stirred for 30 min at ambient temperature and for an additional 60 min at 50° C. The precipitate was filtered off, washed with water (3×25 mL). After drying over anhydrous calcium chloride under high vacuum, 2-chloro-N-(4-cyclopropylmethyl)-N-(6-n-propylamino)[1,3,5]triazine (XXIV) was isolated as a white powder (12 g, 57% yield). LCMS (ESI) m/z=242 $(M+H)^+$.

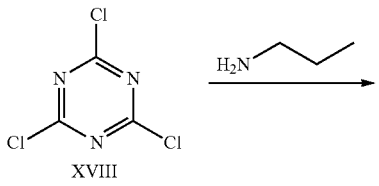
N-(4-Cyclopropylmethyl)-N-(6-n-propylamino) [1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (XXV)

A mixture of 2-chloro-N-(4-cyclopropylmethyl)-N-(6-n-propylamino) [1,3,5]triazine (XXIV) (1.5 g, 6.2 mmol), N,O-dimethylhydroxylamine hydrochloride (3.0 g, 31.0 mmol) and DIPEA (6.5 g, 49.6 mmol) in EtOH (50 mL) was heated at 100° C. for 16 h, after the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (400 mL), washed with water (100 mL), then with a brine solution (100 mL) and dried over Na_2SO_4. The crude was purified by flash column chromatography (pet ether/ethyl acetate=5/1 to 5/2). The solvent was removed under reduced pressure to yield 500 mg (26%) of the desired product. The isolated free amine (500 mg, 1.88 mmol) was dissolved in H_2O (10 mL) and 0.5 M aqueous HCl solution (4.0 mL) was added. The resultant solution was subjected was subjected to lyophilization to yield the desired product, N-(4-cyclopropylmethyl)-N-(6-n-propylamino) [1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (XXV, 520 mg) as a brown oil. LCMS (ESI) m/z=267 $(M+H)^+$. ^1H NMR (500 MHz, DMSO): δ (ppm) 11.80-12.10 (br, 1H), 8.68-8.85 (m, 2H), 3.77 (s, 3H), 3.15-3.36 (m, 7H), 1.49-1.55 (m, 2H), 1.23 (s, 1H), 0.85-0.93 (m, 3H), 0.43-0.49 (m, 2H), 0.22-0.25 (m, 2H).

Example 4

N-(4-Ethylamino)-N-(6-n-propylamino)-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXVII)

Scheme 11



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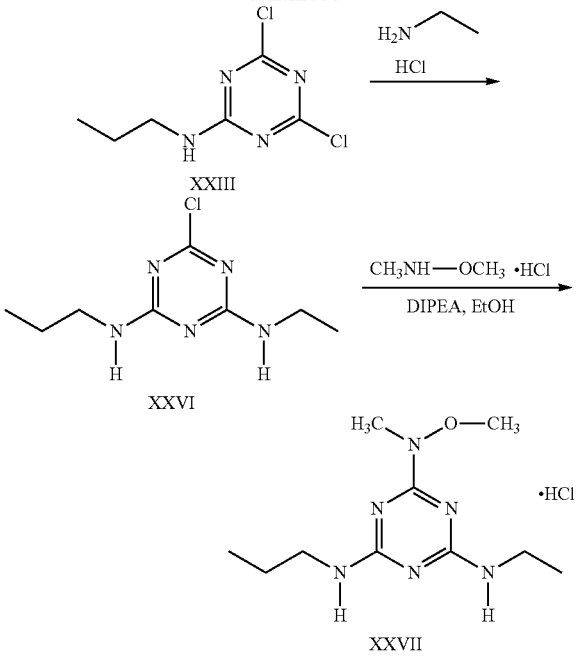


XXIII

XXVI

XXVII

2,4-Dichloro-N-(6-n-propylamino)-[1,3,5]triazine (XXIII)

2,4,6-Trichloro-1,3,5-triazine (XVIII) (20 g, 109 mmol) was dissolved in acetone (100 mL) and poured into ice-water (50 mL) to form a very fine suspension. A solution of propan-1-amine (7.1 g, 120 mmol) in water (20 mL) was added and the temperature maintained at approximately 0° C. To this mixture was added 2 N NaOH (60 mL, 120 mmol) in a dropwise manner to keep the temperature between −5° C. and 0° C. The mixture was then stirred at 0° C. for 60 min. The precipitate was filtered off, washed with water (3×25 mL). After drying over anhydrous calcium chloride under high vacuum, 2,4-dichloro-N-(6-n-propylamino)-[1,3,5]triazine (XVIII) was isolated as a white powder (18 g, 80% yield). LCMS (ESI) m/z=208 $(M+H)^+$.

2-Chloro-N-(4-ethylamino)-N-(6-n-propylamino)-[1,3,5]triazine (XXVI)

2,4-Dichloro-N-(6-n-propylamino)-[1,3,5]triazine (XVIII) (4.0 g, 19.5 mmol) was dissolved in acetone (40 mL) and poured into ice-water (40 mL) to form a very fine suspension. A solution of ethanamine hydrochloride (1.91 g, 23.4 mmol) in water (10 mL) was added and the temperature was maintained at approximately 0° C. A solution of NaOH (2.34 g, 58.5 mmol) in water (10 mL) was added in a dropwise manner to keep the temperature between 0° C. and 5° C. The mixture was then stirred 40 min at room temperature and concentrated. The precipitate was filtered off, washed with water (3×25 mL). After drying over calcium chloride under high vacuum, the desired product, 2-chloro-N-(4-ethylamino)-N-(6-n-propylamino)-[1,3,5]triazine (XXVI) was isolated as a white powder (3.89 g, 92% yield). LCMS (ESI) m/z=216 $(M+H)^+$.

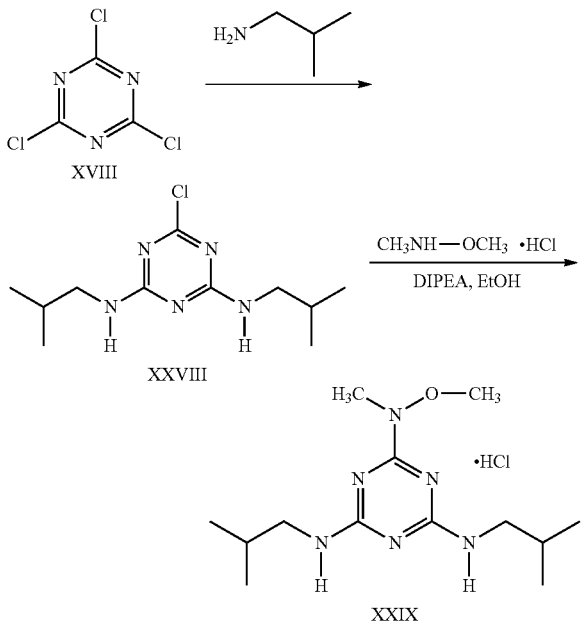
N-(4-Ethylamino)-N-(6-n-propylamino) [1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (XXVII)

A mixture of 2-chloro-N-(4-ethylamino)-N-(6-n-propylamino)-[1,3,5]triazine (XXVI) (2 g, 9.3 mmol), N,O-dimethylhydroxylamine hydrochloride (4.5 g, 46.5 mmol) and DIPEA (8.4 g, 65.1 mmol) in EtOH (20 mL) was heated at 100° C. for 16 h, after which time the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (150 mL), washed with water (100 mL), washed with a brine solution (100 mL) and then dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (pet ether/ethyl acetate=10/1 to 2/1) to yield the desired product (820 mg, 37%). The isolated free amine (820 mg, 3.42 mmol) was dissolved in H_2O (10 mL), and 0.5 M aqueous HCl solution (11 mL) was added. The resultant solution was subjected to lyophilization to yield the desired product, N-(4-ethylamino)-N-(6-n-propylamino)[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (XXVII), as a colorless oil (944 mg). LCMS (ESI) m/z=241 $(M+H)^+$. ^1H NMR (500 MHz, DMSO): δ (ppm) 12.25-12.75 (br, 1H), 8.71-8.75 (m, 2H), 3.75-3.92 (m, 6H), 3.25-3.37 (m, 4H), 1.50-1.55 (m, 2H), 1.09-1.16 (m, 3H), 0.87-0.94 (m, 3H).

Example 5

N-(Bis-4,6-(2-methylpropylamino)) [1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXIX)

Scheme 12



XVIII

XXVIII

XXIX

2-Chloro-N-(4,6-bis-(2-methylpropylamino)-[1,3,5] triazine (XXVIII)

2,4,6-Trichloro-1,3,5-triazine (XVIII) (5.0 g, 27 mmol) was dissolved in acetone (35 mL) and poured into ice-water (50 mL) to form a very fine suspension. A solution of 2-methylpropan-1-amine (4.0 g, 54 mmol) in acetone (20 mL) was added and the temperature was maintained at approximately 0° C. To this mixture, 2 N NaOH (27 mL, 54 mmol) was added in a dropwise manner to keep the temperature between 0° C. and 5° C. The mixture was stirred for 30 min at ambient temperature and for an additional 60 min at 50° C. The pre-

cipitate was filtered off, washed with water (3×25 mL). After drying over calcium chloride under high vacuum, 2-chloro-N-(4,6-bis-(2-methylpropylamino)-[1,3,5]triazine (XXVIII) was isolated as white powder (6.0 g, 87% yield). LCMS (ESI) m/z=258 $(M+H)^+$.

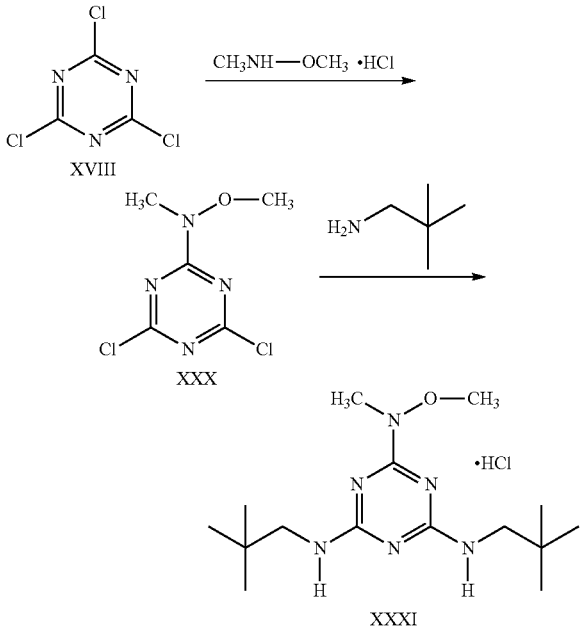
N-(Bis-4,6-(2-methylpropylamino)) [1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (XXIX)

A mixture of 2-chloro-N-(4,6-bis-(2-methylpropylamino)-[1,3,5]triazine (XXVIII) (2.57 g, 10 mmol), N,O-dimethylhydroxylamine hydrochloride (1.94 g, 20 mmol) and DIPEA (5.16 g, 40 mmol) in EtOH (100 mL) was heated at 100° C. for 16 h, after which time the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (200 mL), washed with water (2×100 mL), washed with a brine solution (100 mL) and then dried over Na_2SO_4. The solvent was removed under reduced pressure. The residue was purified by flash column chromatography (pet ether/ethyl acetate=5/1) to yield the desired product (920 mg, 33%). The isolated free amine (920 mg, 3.3 mmol) was dissolved in H_2O (10 mL) and 0.5 M aqueous HCl solution (6.6 mL) was added. The resultant solution was subjected to lyophilization to yield the desired product, N-(bis-4,6-(2-methylpropylamino))[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (XXIX) as a white solid (1.0 g). LCMS (ESI) m/z=283 $(M+H)^+$. ^1H NMR (500 MHz, DMSO): δ (ppm) 12.55-12.60 (br, 1H), 8.57-8.77 (br, 2H), 3.78 (s, 3H), 3.40-3.45 (m, 3H), 3.11-3.19 (m, 4H), 1.80-1.86 (m, 2H), 0.89-0.94 (m, 12H).

Example 6

N-(Bis-4,6-(2,2-dimethylpropylamino)) [1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (XXXI)

Scheme 13



N-(4,6-Dichloro[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXX)

2,4,6-Trichloro-1,3,5-triazine (XVIII) (30 g, 163 mmol) was dissolved in acetone (300 mL), and N,O-dimethylhydroxylamine hydrochloride (15.8 g, 163 mmol) and DIPEA (42 g, 326 mmol) were added and the mixture then stirred at 0° C. for 1 h.

The solution was concentrated and the residue was treated with EtOAc (750 mL), washed with water (100 mL), and the organic layer was dried with Na_2SO_4. The volatiles were removed in vacuo and the residue was purified by flash column chromatography (pet ether/ethyl acetate=50/1 to 10/1) to yield the desired product, N-(4,6-dichloro[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXX), as a white solid (25 g, 73% yield). LCMS (ESI) m/z=210 $(M+H)^+$.

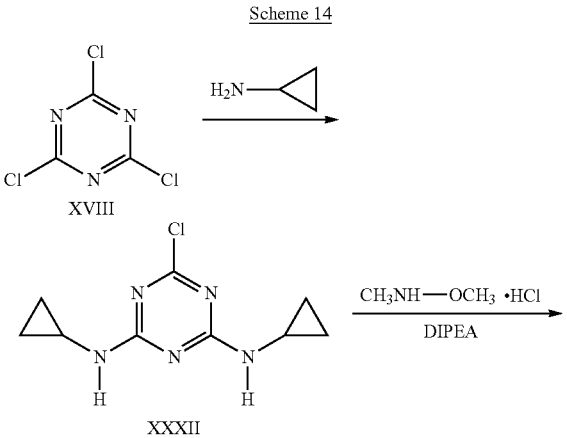
N-(Bis-4,6-(2,2-dimethylpropylamino)) [1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXXI)

A mixture of N-(4,6-dichloro[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXX) (1 g, 4.78 mmol), 2,2-dimethylpropan-1-amine (832.5 mg, 9.57 mmol) and DIPEA (1.85 g, 14.34 mmol) in EtOH (20 mL) was heated at 100° C. for 16 h, after which time the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (40 mL), washed with water (20 mL) and with a brine solution (20 mL), dried over Na_2SO_4, and then concentrated. The crude product was purified by flash column chromatography (pet ether/ethyl acetate=20/1 to 5/1) to yield the desired product (1.4 g, 95%). The isolated free amine (1.4 g, 4.52 mmol) was dissolved in H_2O (10 mL) and 0.5 M HCl solution in H_2O (14.5 mL) was added, and the resultant solution was subjected to lyophilization to yield the desired product, N-(bis-4,6-(2,2-dimethylpropylamino))[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride, as a white solid (1.67 g). LCMS (ESI) m/z=311 $(M+H)^+$. ^1H NMR (500 MHz, DMSO): δ (ppm) 12.40-12.70 (br, 1H), 8.52-8.81 (m, 2H), 3.75-3.79 (m, 3H), 3.33-3.36 (m, 3H), 3.14-3.21 (m, 4H), 0.89-0.96 (m, 18H).

Example 7

4,6-Bis-N-cyclopropylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (XXXIII)

Scheme 14



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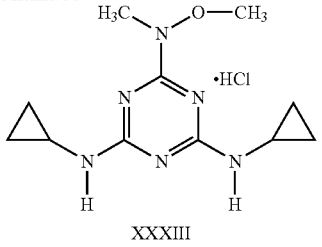


XXXIII

2-Chloro-N-(4,6-bis-(cyclopropylamino)-[1,3,5]triazine (XXXII)

2,4,6-Trichloro-1,3,5-triazine (XVIII) (40 g, 217 mmol) was dissolved in 200 mL of acetone and poured into ice-water (250 mL) to form a very fine suspension. A solution of cyclopropanamine (24.8 g, 435 mmol) was added with stirring at 0° C. To this mixture, 2N NaOH (218 mL, 435 mmol) was added dropwise at a rate to keep the temperature between 0° C. and 5° C. The resultant mixture was stirred for 30 min at ambient temperature and then for an additional 60 min at 50° C. The precipitate was filtered off, washed with water (3×100 mL). After drying over calcium chloride under high vacuum, chloro-N-(4,6-bis-(cyclopropylamino)-[1,3,5]triazine (XXXII) was isolated as a white powder (46 g, 93% yield). LCMS (ESI) m/z=226 $(M+H)^+$.

4,6-Bis-N-cyclopropylamino-[1,3,5]triazin-2-yl)-N, O-dimethyl-hydroxylamine (XXXIII)

A mixture of chloro-N-(4,6-bis-(cyclopropylamino)-[1,3,5]triazine (XXXII) (2.25 g, 10 mmol), N,O-dimethylhydroxylamine hydrochloride (1.94 g, 20 mmol) and DIPEA (5.16 g, 40 mmol) in EtOH (100 mL) was heated at 100° C. for 16 h, and the solvent was then removed under reduced pressure. The residue was dissolved in EtOAc (200 mL), washed with water (2×100 mL) and brine (100 mL) then dried over Na_2SO_4. The solvent was removed under reduced pressure. The residue was purified by flash column chromatography (pet ether/ethyl acetate=3/1) to yield 1.0 g (40%) of the desired product. The isolated free amine (1.0 g, 4.0 mmol) was dissolved in H_2O (10 mL) and 0.5 M aqueous HCl solution (8 mL) and then the solution was lyophilized to yield N-(4,6-bis-cyclopropylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (XXXIII) as a white solid (1.05 g). LCMS (ESI) m/z=251 $(M+H)^+$. ^1H NMR (500 MHz, DMSO): δ (ppm) 12.00-12.80 (br, 1H), 8.70-9.50 (br, 2H), 3.76 (s, 3H), 3.28-3.38 (m, 3H), 2.69-2.89 (m, 2H), 0.59-0.81 (m, 8H).

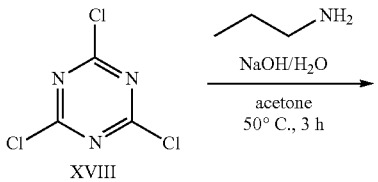
Example 8A

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXXV)

Example 9A

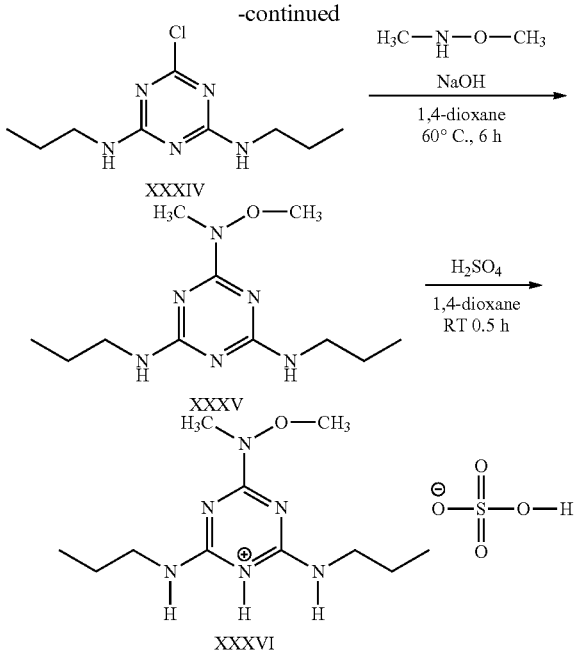
N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrogen sulfate (XXXVI)

Scheme 15A



XVIII

-continued



XXXIV

XXXV

XXXVI

2-Chloro-N-(4,6-bis-(n-propylamino)-[1,3,5]triazine (XXXIV)

A 2 M NaOH solution (82 mL, 162.68 mmol) was added in a dropwise manner to a suspension of 2,4,6-trichloro-1,3,5-triazine (XVIII) (15.00 g, 81.34 mmol) and n-propylamine (13.4 mL, 162.68 mmol) in acetone (300 mL) and water (15 mL) at 0° C. The reaction mixture was heated at 50° C. for 3 h and then cooled. Water (100 mL) was added to the reaction mixture; the resultant precipitate was filtered, washed with water, ethyl ether and dried to yield 2-chloro-N-(4,6-bis-(n-propylamino)-[1,3,5]triazine (XXXIV) (15.88 g, 85% yield).

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXXV)

A mixture of 2-chloro-N-(4,6-bis-(n-propylamino)-[1,3,5]triazine (XXXIV) (10.00 g, 43.53 mmol), N,O-dimethylhydroxylamine hydrochloride (8.49 g, 87.06 mmol) and NaOH (3.13 g, 78.35 mmol) in 1,4-dioxane (120 mL) and water (30 mL) was heated at 60° C. for 6 h, after which the volatiles were removed under reduced pressure. Saturated $NaHCO_3$ solution (500 mL) was added to the residue and the mixture was extracted with EtOAc (3×200 mL). The combined organic extracts were washed with water (300 mL), then with a brine solution (300 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the resultant residue was filtered through silica gel using eluent CH_2Cl_2/EtOH (9/1 v/v) to yield N-(4,6-bis-n-propylamino[1,3,5]triazine-2-yl)-N,O-dimethyl-hydroxylamine (XXXV) (9.96 g, 90% yield).

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrogen sulfate (XXXVI)

Concentrated (95%) H_2SO_4 (0.72 mL, 12.74 mmol) was added in a dropwise manner to a solution of N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXXV, 3.24 g, 12.74 mmol) in 1,4-dioxane (100 mL) at 0° C. The mixture was stirred for 0.5 h at room temperature, volatiles were removed under reduced pressure. The residue

was co-evaporated with dry toluene (3×25 mL). The resulting white residue was crystallized from ethanol/ethyl ether to yield N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine, hydrogen sulfate (XXXVI, 3.86 g, 86% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO): δ (ppm) 12.0-11.2 (1H, br s), 8.7-8.3 (0.7H, br s), 8.10 (0.3H, br s), 7.8-7.3 (1H, m), 3.78 (3H, s), 3.40-3.20 (7H, m), 1.61-1.45 (4H, m), 0.93-0.84 (6H, m). ESI-MS (m/z) 255 $[M+H]^+$; melting point: 134-135° C.

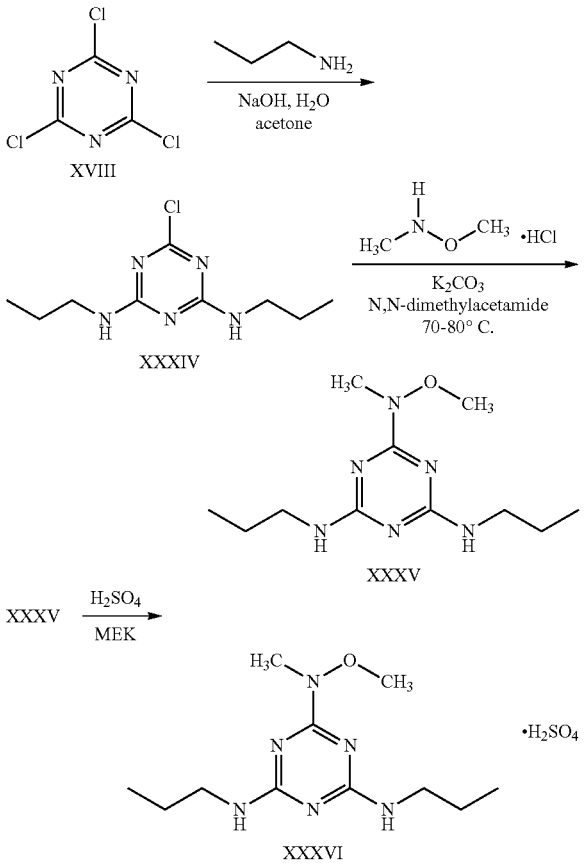
Example 8B

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXXV)

Example 9B

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrogen sulfate (XXXVI)

Scheme 15B



Stage 1: 2-Chloro-N-(4,6-bis-(n-propylamino)-[1,3,5]triazine (XXXIV)

Summary: Di-chloro displacement by 2 equivalents of n-propylamine. In one embodiment, two or more equivalence of n-propylamine produced only the bis-propylamine derivative of the starting material. The reaction progress was monitored by HPLC and the desired intermediate (2-chloro-4,6-bis-n-propylamino-s-triazine) was precipitated. In-process QC Tests were performed.

A suitable glass reactor vessel equipped with a mechanical stirrer, thermocouple, condenser, addition funnel and a temperature control mantle was charged with 8 L of acetone followed by 1 kg (5.42 moles) of cyanuric chloride. The stirring mixture was pre-cooled to 15° C. and n-propylamine (at ambient temperature) was added slowly via addition funnel, to maintain the temperature below 45° C. A 2M NaOH solution was prepared and was added to the mixture at a rate to maintain the temperature below 45° C. The pH of the mixture was acidic (approximately pH=4) and 6N NaOH was added to adjust the pH to 8-9. The mixture was stirred at 40-50° C. for 0.5 h and the reaction was monitored for completion by IPC HPLC analysis. The reaction was deemed complete when <2% of cyanuric chloride was detected. Analysis was repeated every hour until reaction was complete.

After reaction was complete, WFI (sterile water) was added slowly to maintain temperature below 50° C. The resulting suspension was allowed to cool to room temperature while stirring overnight. The solids were filtered through a polypropylene filter cloth and washed with acetone/water (1:2) followed by 1.5 L of MTBE. The solids were dried on the filter (assisted by vacuum) then placed in a vacuum oven (45±5° C., >29" Hg) for a minimum of 6 h to give <0.5% in change in weight loss. A sample was collected for analytical testing (QC HPLC analysis and Karl Fisher analysis); 2 g was collected for QA retain. The product produced in Stage 1 was a white solid. This powder was transferred from drying pans to poly bag with nylon tie, double bagged and placed into fiber drum; label and submit to QA for quarantine storage.

Stage 2, Step 1: N-(4,6-Bis-n-propylamino-[1,3,5] triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXXV)

Summary: Chloro displacement by 1 equivalent of N-methoxymethylamine. The reaction progress was monitored by HPLC and precipitation of desired product free base was accomplished by adding water and cooling. In-process QC Tests were pre-formed. The free base was converted to the corresponding sulfate salt with crystallization and the final product was subjected to vacuum oven drying. In-process QC Tests were performed as well as finished product QC testing.

A suitable round-bottom flask equipped with a mechanical stirrer, thermocouple, condenser and a heating mantle was charged with 6 L of N,N-dimethylacetamide (DMA) followed by 1 kg (4.35 moles) of 6-chloro-N,N-dipropyl-[1,3,5]-triazine-2,4-diamine (Stage 1 Product). Added to this stirring mixture at room temperature was K_2CO_3 (1.2 kg, 6.53 moles, 2 eq.), with rinsing with a small quantity of additional DMA. To this, was added N,O-dimethylhydroxylamine hydrochloride (0.637 kg, 8.71 moles, 1.5 eq.) in portions over ~5-10 minutes to reduce foaming (and while maintaining the temperature below 60° C.) with rinsing using a small quantity of additional DMA. The mixture was heated to 75-80° C. and stir for a minimum of 0.5 hours. Once at 75-80° C., the reaction was monitored for completion by HPLC. The mixture was cooled to below 65° C. and water (12 L) was added. The resulting suspension was allowed to cool to room temperature while stirring overnight (18 h). The resultant solids were filtered and washed with 1.2 L of water. The filter cake to air-dry one hour and a sample for OVI amine GC analysis was obtained. The remaining solids were vacuum oven dried (45° C., >29" Hg) for a minimum of 6 hours (NMT 1% weight change). The desired product (free base (XXXV)) was a dense white solid. An IPC sample for testing was obtained.

Crystalline N-(4,6-Bis-n-propylamino-[1,3,5]-triazin-2-yl)-N,O-dimethyl-hydroxylamine free base (XXXV)

A solution of (XXXV) free base in methylene chloride was rapidly evaporated to dryness. The resulting solid was dried under vacuum at ca. 25° C. for ca. 45 minutes (XXXV free-base Form A). DSC: Sharp endotherm at ca. 95.2° C. XRPD: FIG. **22**.

Stage 2, Step 2: Salt Formation and Crystallization of N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrogen sulfate (XXXVI)

Methyl ethyl ketone (20 L) was added to 2,800 g (12.2 moles) of substrate free base (XXXV). The mixture was stirred and heated to 40° C. then polish filtered into a 72-L reactor through a medium porosity sintered glass funnel to yield a clear solution and a fluffy white residue (12 g, 0.43% of the free base weight). The mixture was warmed to 45° C. and concentrated H_2SO_4 (12.8 moles, 1.05 eq. based on pre-filtered weight) was added slowly over 1 h via addition funnel. The mixture was allowed to stir overnight (18 h) while cooling (A solid precipitate was observed to begin forming at approximately 36° C.; final temp=27° C.).

The mixture was further cooled to 15° C., stirred 0.5 h and filtered. The solid product cake was washed with methyl ethyl ketone (6 L), air dried on the Büchner funnel (vacuum assisted) for 4 h, then placed in a vacuum oven (>29" Hg, 50-55° C.) and dried for at least 6 h to give 3,252 g (84%) of N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrogen sulfate (XXXVI) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.88 (quin., 6H), 1.41-1.63 (m, 4H), 3.16-3.40 (m, 7H), 3.69-3.83 (m, 3H), 7.50-8.56 (m, 2H). In Process Purity (HPLC (AUC @ 210 nm)=99.81%. Filter residue: ^{1}H NMR (400 MHz, methanol-d_4) 3.27-3.35 (m), 1.55-1.65 (q) 0.90-0.95 (t). MS m/z: 212 [M+H], consistent with data N-(4,6-bis(propylamino)-1, 3,5-triazin-2-ol (CLXXVIII) (c.f., Comparative Example 1). The isolated title compound contained no detectable (CLXXVIII).

Stage 1:

IPC Test 1: Temperature Chart Recording during additions—
Maintain temperature control specified in process steps

IPC Test 2: IPC HPLC analysis—
Reaction is complete when <2% of cyanuric chloride is detected.
Repeat analysis every hour until reaction is complete.
Contact supervisor if reaction is not complete after third sample.

Stage 1 Product QC testing:
QC HPLC analysis—
Record results of assay of cyanuric chloride and 2-chloro-4,6-bis-n-propylamino-s-triazine
Karl Fisher—
Record results

Stage 2:

Step 1: Reaction:

IPC Test 1: Temperature Chart Recording during additions—
Maintain temperature control specified in process steps

IPC Test 2: IPC HPLC analysis—
Reaction is complete when <2% of 2-chloro-4,6-bis-n-propylamino-s-triazine is detected.
Repeat analysis every hour until reaction is complete.
Contact supervisor if reaction is not complete after third sample.

IPC Test 3: Residual amine analysis (by GC)—
n-Propylamine and N,O-dimethylhydroxylamine levels NMT 0.1%

IPC Test 4: Weight Change during drying—
NMT 1%

IPC Test 5: IPC HPLC analysis—
Purity by HPLC (AUC) record results

Step 2: Salt Formation and Crystallization

IPC Test 1: Temperature Chart Recording during concentrated sulfuric acid addition—
Maintain temperature control specified in process step

IPC Test 2: OVI of MEK and DMAc by GC—
NMT 800 ppm each

IPC Test 4: Weight Change during drying—
NMT 1%

Proton Nuclear Magnetic Resonance (NMR) Spectroscopy

^{1}H NMR data of N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrogen sulfate (XXXVI) was obtained as a solution in DMSO-d_6 at 400 MHz and is presented in FIG. **10** and shift assignments are presented in Table 1.

TABLE 1

^{1}H Chemical shift assignments for (XXXVI) in DMSO-d_6 at 25° C.

Resonance Peak	δ^{1}H (ppm)	Assignment
1	0.88	13, 17
2	1.53	12, 16
3	3.30	11, 15
4	3.35	18
5	3.77	9
6	[7.42, 8.70]	10, 14

Carbon-13 NMR Spectroscopy

^{13}C NMR data of N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrogen sulfate (XXXVI) was obtained as solutions in DMSO-d_6 at 100 MHz and is presented in FIG. **11** and shift assignments are presented in Table 2.

TABLE 2

^{13}C Chemical shift assignments for (XXXVI) in DMSO-d_6 at 25° C.

Resonance peak	δ^{13}C (ppm)	Assignment
1	11.25	13, 17
2	21.88	12, 16
3	34.08	18
4	42.24	11, 15
5	61.86	9
6	154.63	2
7	155.79	4, 6

Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR spectrum of N-(4,6-bis-n-propylamino-[1,3,5] triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrogen sulfate (XXXVI) is presented in Table 3 and FIG. **12**.

TABLE 3

FTIR spectrum of (XXXVI)

Wavenumber (cm^{-1})	Assignment
3284	N—H stretch
2850-2960	C—H stretch

TABLE 3-continued

FTIR spectrum of (XXXVI)

Wavenumber (cm^{-1})	Assignment
1615-1700	C═N bend
1536-1656	N—H bend
1020-1340	C—N stretch

High and Low Resolution Mass Spectrometry

The mass obtained from Liquid Chromatography-Mass Spectrometry (LCMS) was 254 amu, which agrees with the theoretical mass of N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrogen sulfate (XXXVI). High-resolution results obtained through direct injection are presented in Table 4.

TABLE 4

Mass spec results for (XXXVI)

Calculated Molecular Weight	Theoretical Exact Mass	Formula
254.1863	254.1855	$C_{11}H_{22}N_6O$

Chromatographic Purity

The HPLC chromatographic purity of the RS was determined to be 100.0% by area, with no related substances detected.

Water by Determination

The water content was determined to be 0.04% by Karl Fischer titration.

Elemental Analysis

Elemental analysis of N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrogen sulfate (XXXVI) was obtained and is presented in Table 5.

TABLE 5

Elemental analysis results for (XXXVI)

Element	Theoretical (%)	Result (%)
C	37.49	37.59
H	6.86	6.95
N	23.85	23.86
S	9.10	9.08

Thermal Analysis by Differential Scanning Calorimetry (DSC)

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrogen sulfate (XXXVI) was analyzed from 25° C. to 250° C., at a rate of 10° C. per minute as per cUSP <891>/EP 2.2.34 and was found to have endotherms at 90.19° C., 126.38° C., and 138.30° C. FIG. **13**.

X-ray Powder Diffraction

XRPD diffraction pattern of N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrogen sulfate (XXXVI) was obtained and is consistent with Form A; presented in FIG. **14**.

Counterion Content

N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrogen sulfate (XXXVI) was found to contain 27.13% sulfate content by titration.

pH of Aqueous Solution

A 1% aqueous solution of N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrogen sulfate (XXXVI) yielded a pH of 1.89.

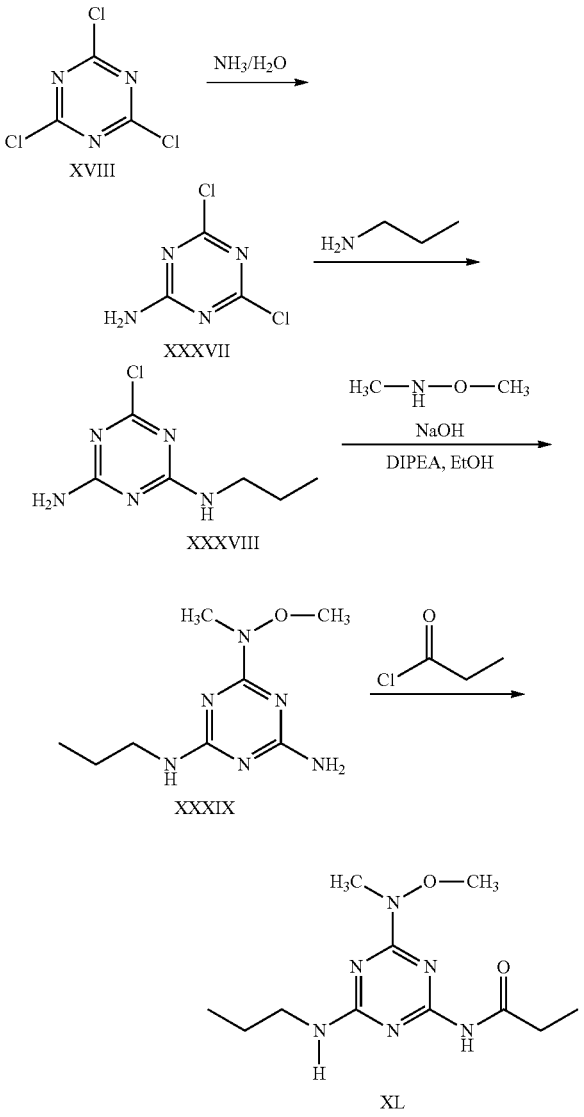
Physical Description

N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrogen sulfate (XXXVI) was determined to be a white crystalline solid.

Example 10

N-(4-(Methoxy(methyl)amino)-6-(propylamino)-1,3,5-triazin-2-yl)propionamide (XL)

Scheme 16



6-Amino-2,4-dichloro-[1,3,5]triazine (XXXVII)

2,4,6-Trichloro-1,3,5-triazine (XVIII) (10.0 g, 55 mmol) was dissolved in acetone (80 mL) and poured into ice-water (80 mL) to form a very fine suspension. To this mixture, 1 N ammonium hydroxide solution (108 mL, 109.4 mmol) was added at 0° C. The reaction was stirred for 30 min at ambient temperature and for additional 60 min at 25° C. The precipitate was filtered off, washed with water (3×25 mL). After

drying over calcium chloride under high vacuum, 6-amino-2,4-dichloro-[1,3,5]triazine (XXXVII) was isolated as white powder (7.4 g, 82% yield). LCMS (ESI) m/z=165 $(M+H)^+$.

6-Amino-2-chloro-4-n-propylamino-[1,3,5]triazine (XXXVIII)

6-Amino-2,4-dichloro-[1,3,5]triazine (XXXVII) (30.0 g, 187 mmol) was dissolved in acetone (100 mL) and poured into ice-water (100 mL) to form a very fine suspension. To this mixture, a solution of propan-1-amine (11.0 g, 187 mmol) in acetone (20 mL) was added at 0° C. To this reaction, 2 N NaOH (94 mL, 187 mmol) was added dropwise at a rate to keep the temperature between 0° C. and 5° C. The mixture was stirred for 30 min at ambient temperature and for an additional 60 min at 50° C. The mixture was concentrated and then the precipitate was filtered off and washed with water (3×100 mL). After drying over calcium chloride under high vacuum, 6-amino-2-chloro-4-n-propylamino-[1,3,5]triazine (XXXVIII) was isolated as a white powder (35 g, 100% yield). LCMS (ESI) m/z=188 $(M+H)^+$.

N-(6-Amino-4-n-propylamino-[1,3,5]triazin-2-yl)-N, O-dimethyl-hydroxylamine (XXXIX)

A mixture of 6-amino-2-chloro-4-n-propylamino-[1,3,5] triazine (XXXVIII) (5 g, 26.65 mmol), N,O-dimethylhydroxylamine hydrochloride (13 g, 133.24 mmol) and DIPEA (27.5 g, 213.2 mmol) in EtOH (100 mL) was heated at 100° C. for 16 h, after which time the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (400 mL), which was washed with water (200 mL) and then with a brine solution (200 mL) and finally dried over Na_2SO_4. The solvent was removed under reduced pressure to yield N-(6-amino-4-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXXIX) as a white solid (5 g, 89% yield). LCMS (ESI) m/z=213 $(M+H)^+$.

N-(4-(Methoxy(methyl)amino)-6-(propylamino)-1,3, 5-triazin-2-yl)propionamide (XL)

N-(6-amino-4-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXXIX) (5 g, 23.58 mmol) was dissolved in THF (50 mL). Propionyl chloride (3.25 g, 35.38 mmol) and DIPEA (5.47 g, 42.44 mmol) were added at 0° C. The resultant mixture was stirred at ambient temperature for 10 min, then stirred at 70° C. for 16 h, after which time the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (250 mL), and this extract was washed with water (80 mL) and then with a brine solution (80 mL), and lastly dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (pet ether/ethyl acetate=5/1 to 2/1) to yield 930 mg (15%) of the desired product. The isolated free amine (930 mg, 3.47 mmol) was dissolved in H_2O (10 mL) and 0.5 M HCl solution in H_2O (10.4 mL) and then the solution was lyophilized to yield N-(4-(methoxy(methyl) amino)-6-(n-propylamino)-1,3,5-triazin-2-yl)propionamide (XL) as a colorless oil (1.06 g). LCMS: (ESI) m/z=269 $(M+H)^+$. ^1H NMR (500 MHz, DMSO): δ (ppm) 12.23 (s, 1H), 8.25-9.45 (m, 2H), 3.76-3.84 (m, 3H), 3.30-3.44 (m, 5H), 2.55-2.56 (m, 1H), 2.21-2.22 (m, 1H), 1.53-1.58 (m, 2H), 0.88-1.08 (m, 6H).

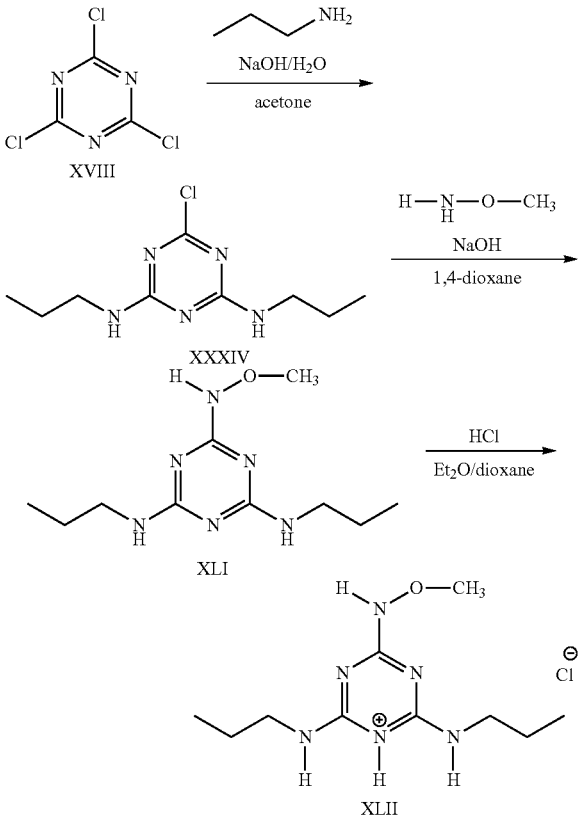
Example 11

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-methyl-hydroxylamine (XLI)

Example 12

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-methyl-hydroxylamine hydrochloride (XLII)

Scheme 17



2-Chloro-N-(4,6-bis-(n-propylamino)-[1,3,5]triazine (XXXIV)

A 2 M NaOH solution (163 mL, 325.36 mmol) was added in a dropwise manner to a suspension of 2,4,6-trichloro-1,3, 5-triazine (XVIII) (30.0 g, 162.68 mmol) and n-propylamine (26.8 mL, 325.36 mmol) in acetone (600 mL) and water (30 mL) at 0° C. (water—ice/NaCl bath). The ice bath was removed and the reaction mixture was heated at 50° C. for 3 h, then cooled. Water (200 mL) was added to the reaction mixture; the precipitate was filtered, washed with water (200 mL) and dried over P_2O_5 at 40° C. for 20 h to yield 2-chloro-N-(4,6-bis-(n-propylamino)-[1,3,5]triazine (XXXIV, 33.6 g, 90% yield). 400 MHz ^1H NMR (DMSO-d_6, ppm) 7.80 (0.85H, t, J=5.5 Hz), 7.76-7.66 (1H, m), 7.49 (0.15H, t, J=5.5

Hz), 3.22-3.11 (4H, m), 1.55-1.42 (4H, m), 0.88-0.82 (6H, m). ESI-MS (m/z): 230, 232 $[M+H]^+$.

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-methyl-hydroxylamine (XLI)

A mixture of 6-chloro-N,N'-dipropyl-[1,3,5]triazine-2,4-diamine (XXXIV) (2.30 g, 10.01 mmol), O-methyl-hydroxylamine hydrochloride (1.67 g, 20.02 mmol) and NaOH (0.72 g, 18.00 mmol) in 1,4-dioxane (30 mL) and water (6 mL) was heated at 60° C. for 3 h. After this time, NaOH (0.72 g, 18.00 mmol) was added and the reaction mixture was heated for another 3 h. The volatiles were removed under reduced pressure. Saturated $NaHCO_3$ solution (100 mL) was added to the residue, the mixture was extracted with EtOAc (3×25 mL). The combined organic extracts were washed with water (50 mL), brine (50 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography using gradient elution from CH_2Cl_2/EtOH (99:1) to CH_2Cl_2/EtOH (95:5) to yield 2.17 g (90%) of N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-O-methyl-hydroxylamine (XLI). ESI-MS (m/z): 241 $[M+H]^+$

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-methyl-hydroxylamine hydrochloride (XLII)

A 2M HCl/ethyl ether (4.5 mL, 9.00 mmol) was added to the solution N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-O-methyl-hydroxylamine (XLI) (2.17 g, 9.03 mmol) in 1,4-dioxane (5 mL) at 0° C. The mixture was stirred for 0.5 h at 0° C., volatiles were removed under reduced pressure to yield N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-O-methyl-hydroxylamine hydrochloride (XLII) in quantitative yield. 400 MHz ^{1}H NMR (DMSO-d_6, ppm) 12.5-11.5 (2H, br s), 8.49 (1H, br s), 8.34 (1H, br s), 3.71 (3H, s), 3.34-3.16 (4H, m), 1.59-1.46 (4H, m), 0.94-0.83 (6H, m). ESI-MS (m/z) 241 $[M+H]^+$.

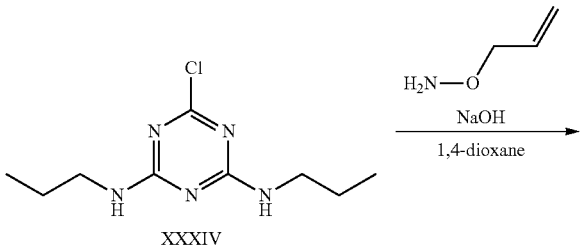
Example 13

O-Allyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-hydroxylamine (XLIII)

Example 14

O-Allyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-hydroxylamine hydrochloride salt (XLIV)

Scheme 18



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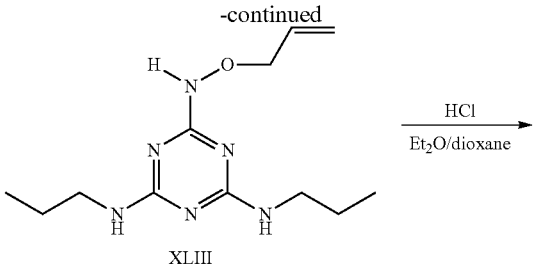
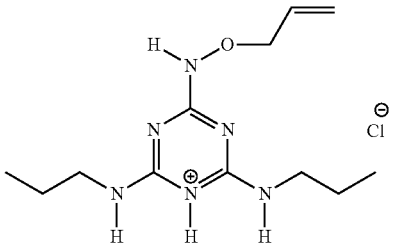


XLIII



XLIV

O-Allyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-hydroxylamine

A mixture of 2-chloro-N-(4,6-bis-(n-propylamino)-[1,3,5]triazine (XXXIV) (2.00 g, 8.71 mmol), O-allyl-hydroxylamine hydrochloride (1.91 g, 17.42 mmol) and NaOH (0.70 g, 17.42 mmol) in 1,4-dioxane (25 mL) and water (5 mL) was heated at 60° C. for 4 h. The volatiles were removed under reduced pressure. Saturated $NaHCO_3$ solution (100 mL) was added to the residue and the mixture was extracted with EtOAc (3×25 mL). The combined organic extracts were washed with water (50 mL), brine (50 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography using gradient elution from CH_2Cl_2/EtOH (99:1) to CH_2Cl_2/EtOH (95:5) to yield O-allyl-N-(4,6-bis-propylamino-[1,3,5]triazin-2-yl)-hydroxylamine (XLIII, 2.05 g, 88% yield). ESI-MS (m/z) 267 $[M+H]^+$.

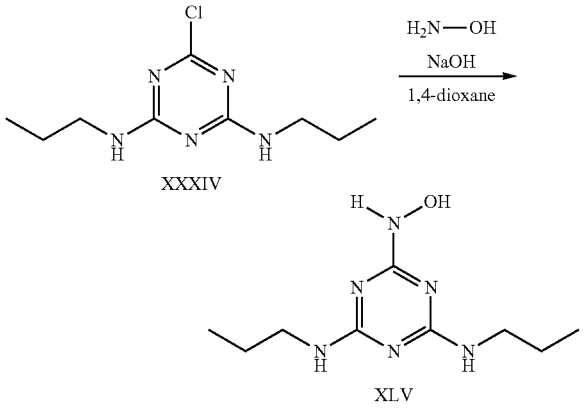
O-Allyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-hydroxylamine hydrochloride

O-Allyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-hydroxylamine hydrochloride (XLIV) was prepared from O-allyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-hydroxylamine (XLIII) and 2M HCl/ethyl ether as described in Example 12. 400 MHz ^{1}H NMR (DMSO-d_6, ppm) 11.7-10.0 (1H, m), 7.9-7.1 (2H, m), 6.09-5.92 (1H, m), 5.39-5.18 (2H, m), 4.35 (2H, d, J=6.0 Hz), 3.28-3.11 (4H, m), 1.56-1.42 (4H, m), 0.91-0.81 (6H, m). ESI-MS (m/z): 267 $[M+H]^+$. MP: 130-132° C.

Example 15

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-hydroxylamine (XLV)

Scheme 19



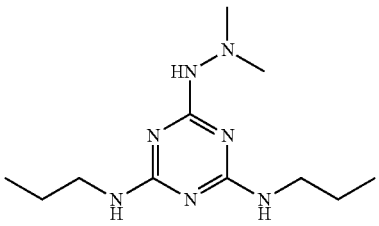
N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-hydroxylamine (XLV) was prepared from 2-chloro-N-(4,6-bis-(n-propylamino)-[1,3,5]triazine (XXXIV) and hydroxylamine hydrochloride as described in Example 13 (99% yield). 400 MHz ^{1}H NMR (DMSO-d_6, ppm) 9.0-8.6 (1H, br s), 8.39-8.14 (1H, s), 6.89-6.55 (2H, m), 3.23-3.06 (4H, m), 1.54-1.40 (4H, m), 0.84 (6H, t, J=7.4 Hz). ESI-MS (m/z): 227 $[M+H]^+$. MP: 138-141° C.

Example 16

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N',N'-dimethylhydrazine (XLVI)

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N',N'-dimethylhydrazine (XLVI) may be prepared from 2-chloro-N-(4,6-bis-(n-propylamino)-[1,3,5]triazine (XXXIV) and N,N-dimethylhydrazine as described in Example 19.

Scheme 20

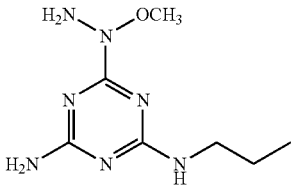


XLVI

Example 17

6-(Methoxy(methyl)amino)-N2-propyl-1,3,5-triazine-2,4-diamine (XLVII)

Scheme 21

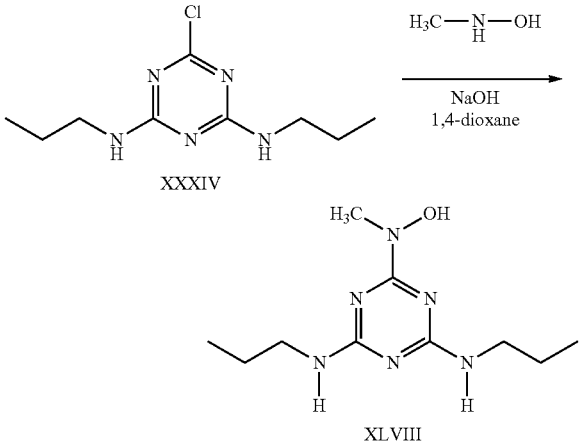


6-(Methoxy(methyl)amino)-N2-propyl-1,3,5-triazine-2,4-diamine (XLVII) may be prepared from 6-amino-2-chloro-4-n-propylamino-[1,3,5]triazine (XXXVIII) and N,O-dimethylhydroxylamine as described in Example 10.

Example 18

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N-methyl-hydroxylamine (XLVIII)

Scheme 22



N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N-methyl-hydroxylamine (XLVIII) was prepared from 2-chloro-N-(4,6-bis-(n-propylamino)-[1,3,5]triazine (XXXIV) and N-methyl-hydroxylamine hydrochloride as described in Example 13 (90% yield). 400 MHz ^{1}H NMR (DMSO-d_6, ppm) 8.93 (1H, s), 6.92-6.43 (2H, m), 3.23-3.07 (7H, m), 1.55-1.38 (4H, m), 0.84 (6H, t, J=7.4 Hz). ESI-MS (m/z) 241 $[M+H]^+$.

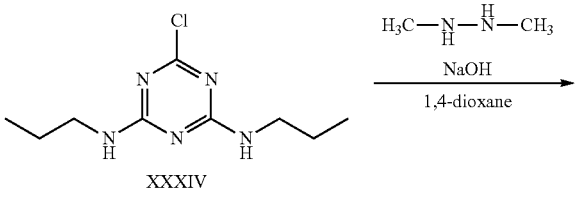
Example 19

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,N'-dimethyl-hydrazine (XLIX)

Example 20

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,N'-dimethyl-hydrazine hydrogen sulfate

Scheme 23



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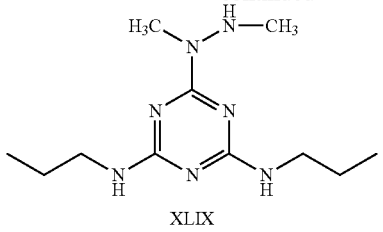
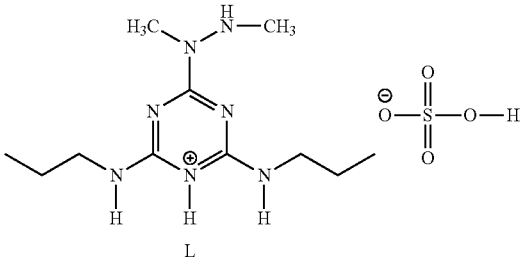


XLIX



L

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,N'-dimethyl-hydrazine (XLIX)

A mixture of 2-chloro-N-(4,6-bis-(n-propylamino)-[1,3,5] triazine (XXXIV) (2.50 g, 10.88 mmol), N,N'-dimethyl-hydrazine dihydrochloride (2.89 g, 21.76 mmol) and NaOH (2.18 g, 54.40 mmol) in 1,4-dioxane (40 mL) and water (20 mL) was heated at 60° C. for 18 h. The volatiles were removed under reduced pressure. Saturated $NaHCO_3$ solution (100 mL) was added to the residue, the mixture was extracted with EtOAc (3×50 mL). The combined organic extracts were washed with water (75 mL), brine (75 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography using gradient elution (CH_2Cl_2/EtOH (99:1) to CH_2Cl_2/EtOH (95:5)) to yield N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,N'-dimethyl-hydrazine (1.17 g, 42%). 200 MHz ^1H NMR (DMSO-d_6, ppm): 6.81-6.44 (2H, m), 5.31 (1H, br s), 3.24-3.08 (4H, m), 3.05 (3H, s), 2.47-2.40 (3H, m), 1.57-1.37 (4H, m), 0.84 (6H, t, J=7.4 Hz). ESI-MS (m/z): 254 $[M+H]^+$.

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,N'-dimethyl-hydrazine hydrogen sulfate (L)

95% H_2SO_4 (0.26 mL, 4.62 mmol) was added dropwise to the solution of 6-(N,N'-dimethyl-hydrazino)-N,N'-dipropyl-[1,3,5]triazine-2,4-diamine (XLIX) (1.17 g, 4.62 mmol) in 1,4-dioxane (10 mL) at 0° C. The mixture was stirred for 0.5 h at room temperature; volatiles were removed under reduced pressure. The residue was co-evaporated with dry toluene (3×25 mL) to yield N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,N'-dimethyl-hydrazine hydrogen sulfate (L) in quantitative yield. 400 MHz ^1H NMR (DMSO-d_6, ppm) 8.48-8.32 (1H, m), 7.9-7.7 (0.5H, br s), 7.70-7.61 (0.5H, m), 3.34-3.20 (4H, m), 3.21 (1.5H, s), 3.17 (1.5H, s), 2.52 (1.5H, s), 2.51 (1.5H, s, overlapped with DMSO), 1.59-1.46 (4H, m), 0.93-0.82 (6H, m). ESI-MS (m/z): 254 $[M+H]^+$.

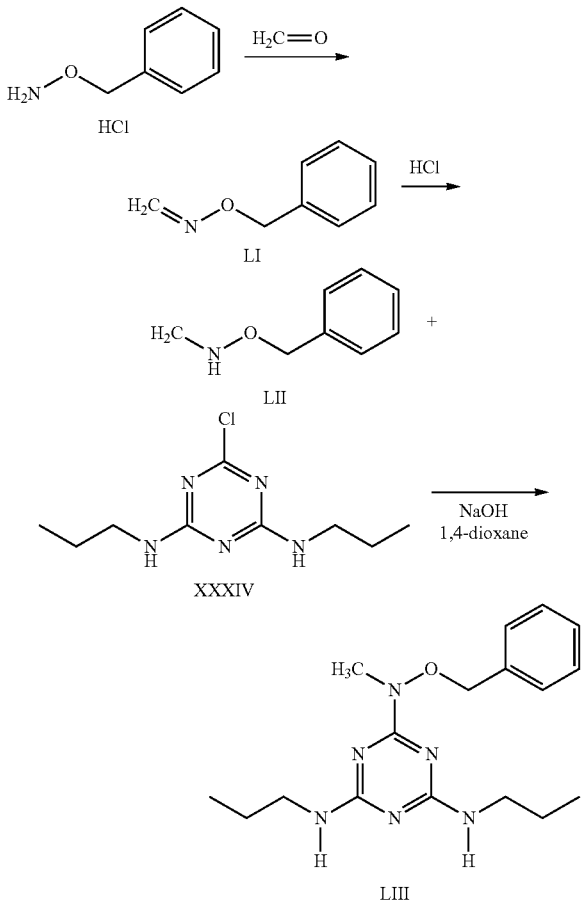
Example 21

O-Benzyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N-methyl-hydroxylamine (LIII)

Example 22

O-Benzyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N-methyl-hydroxylamine hydrogen sulfate salt (LIV)

Scheme 24



HCl

LI

LII

XXXIV

LIII

Formaldehyde O-benzyl-oxime (LI)

A NaOH (1.25 g, 31.32 mmol) solution in water (6 mL) was added to the mixture of O-benzyl-hydroxylamine hydrochloride (5.00 g, 31.32 mmol) and formaldehyde (~37 wt. % in H_2O) (2.3 mL, 31.32 mmol) in toluene (40 mL). The reaction mixture was stirred at room temperature for 1 h. After this time, the organic phase was separated and the water phase was extracted with dichloromethane (3×30 mL). The combined organic phases were dried over Na_2SO_4, and concentrated in vacuo to yield formaldehyde O-benzyl-oxime (LI, 4.15 g, 98%). 400 MHz ^1H NMR ($CDCl_3$, ppm) 7.40-7.29 (5H, m), 7.09 (1H, d, J=8.2 Hz), 6.47 (1H, d, J=8.2 Hz), 5.14 (2H, s).

O-Benzyl-N-methyl-hydroxylamine (LII)

A 1M HCl/EtOH solution (50 mL) was added dropwise to the solution of formaldehyde O-benzyl-oxime (3.85 g, 28.48 mmol) in EtOH at 0° C. The mixture was stirred at room temperature for 1 hour, volatiles were removed in vacuo. The residue was dissolved in dichloromethane (100 mL), washed with saturated $NaHCO_3$ solution (75 mL), water (75 mL), and dried over Na_2SO_4. The product was purified by flash column chromatography using gradient elution from petroleum ether/EtOAc (9:1) to petroleum ether/EtOAc (7:1) to yield O-benzyl-N-methyl-hydroxylamine (1.72 g, 44%). 200 MHz ^1H NMR ($CDCl_3$, ppm) 7.40-7.27 (5H, m), 5.53 (1H, br s), 4.71 (2H, s), 2.73 (3H, s).

O-Benzyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N-methyl-hydroxylamine (LIII)

2-Chloro-N-(4,6-bis-(n-propylamino)-[1,3,5]triazine (XXXIV) and O-benzyl-N-methyl-hydroxylamine (LII) were reacted as described in Example 13 to yield O-benzyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N-methyl-hydroxylamine (LIII) (29% yield). 200 MHz ^1H NMR (DMSO-d_6, ppm) 7.52-7.28 (5H, m), 7.07-6.67 (2H, m), 4.93 (2H, s), 3.26-3.03 (7H, m), 1.58-1.39 (4H, m), 0.85 (6H, t, J=7.2 Hz). ESI-MS (m/z): 331 $[M+H]^+$.

O-Benzyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N-methyl-hydroxylamine hydrogen sulfate (LIV)

O-Benzyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N-methyl-hydroxylamine (LIII) was reacted with 95% H_2SO_4 as described in Example 20 to yield O-benzyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N-methyl-hydroxylamine hydrogen sulfate (LIV) in quantitative yield. 400 MHz ^1H NMR (DMSO-d_6, ppm) 12.0-10.9 (1H, br s), 8.7-8.3 (1H, br s), 7.56-7.46 (2H, m), 7.46-7.37 (2.5H, m), 7.36-7.30 (0.5H, m), 5.07-4.95 (2H, m), 3.44-3.16 (7H, m), 1.61-1.45 (4H, m), 0.94-0.82 (6H, m). ESI-MS (m/z): 331 $[M+H]^+$.

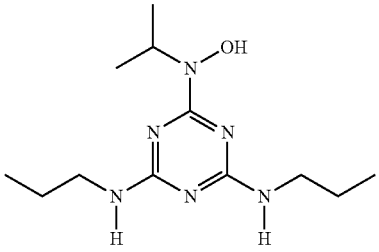
Example 23

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N-isopropyl-hydroxylamine (LV)

Example 24

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N-isopropyl-hydroxylamine hydrogen sulfate (LVI)

Scheme 25



LV

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N-isopropyl-hydroxylamine (LV)

2-Chloro-N-(4,6-bis-(n-propylamino)-[1,3,5]triazine (XXXIV) and N-isopropyl-hydroxylamine hydrochloride were reacted as described in Example 13 to yield N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N-isopropyl-hydroxylamine (LV) (61% yield). ESI-MS (m/z): 269 $[M+H]^+$.

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N-isopropyl-hydroxylamine hydrogen sulfate (LVI)

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N-isopropyl-hydroxylamine hydrogen sulfate (LVI) was prepared from N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N-isopropyl-hydroxylamine (LV) and 95% H_2SO_4 as described in Example 20 (95% yield). 200 MHz ^1H NMR (DMSO-d_6, ppm) 11.5-11.1 (1H, br s), 10.66-10.40 (1H, m), 8.45 (1H, s), 7.75-7.36 (1H, m), 4.77-4.55 (1H, m), 3.30-3.16 (4H, m), 1.61-1.44 (4H, m), 1.17 (6H, t, J=7.0 Hz), 0.89 (3H, t, J=7.3 Hz), 0.86 (3H, t, J=7.3 Hz). ESI-MS (m/z) 269 $[M+H]^+$. M.P.: 154-156° C.

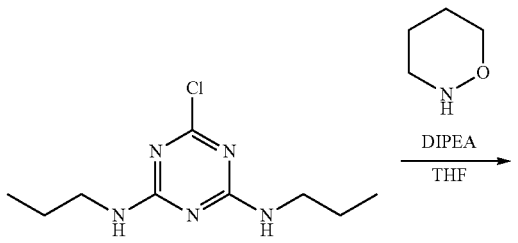
Example 25

6-[1,2]Oxazinan-2-yl-N,N'-dipropyl-[1,3,5]triazine-2,4-diamine (LVII)

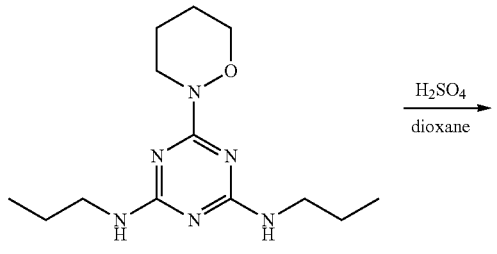
Example 26

6-[1,2]Oxazinan-2-yl-N,N'-di-n-propyl-[1,3,5]triazine-2,4-diamine hydrogen sulfate (LVIII)

Scheme 26



XXXIV



LVII

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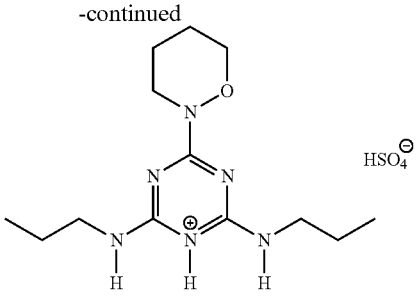


LVIII

6-[1,2]Oxazinan-2-yl-N,N'-di-n-propyl-[1,3,5]triazine-2,4-diamine (LVII)

An ACE® pressure tube was charged with 2-chloro-N-(4,6-bis-(n-propylamino)-[1,3,5]triazine (XXXIV) (1.50 g, 6.53 mmol), N-ethyldiisopropylamine (19.59 mmol), 1,2-oxazinane hydrochloride (1.61 g, 13.06 mmol) and tetrahydrofuran. The reaction mixture was heated at 100° C. for 2 h, then cooled and poured into saturated $NaHCO_3$ solution (50 mL). The suspension was extracted with EtOAc (3×25 mL). The combined organic extracts were washed with water (50 mL), brine (50 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography using gradient elution from CH_2Cl_2/EtOH (99:1) to CH_2Cl_2/EtOH (95:5) to yield 6-[1,2]oxazinan-2-yl-N,N'-di-n-propyl-[1,3,5]triazine-2,4-diamine (LVII) (1.63 g, 89%). ESI-MS (m/z): 281 $[M+H]^+$.

6-[1,2]Oxazinan-2-yl-N,N'-di-n-propyl-[1,3,5]triazine-2,4-diamine hydrogen sulfate (LVIII)

6-[1,2]Oxazinan-2-yl-N,N'-di-n-propyl-[1,3,5]triazine-2,4-diamine hydrogen sulfate (LVIII) was prepared from 6-[1,2]oxazinan-2-yl-N,N'-di-n-propyl-[1,3,5]triazine-2,4-diamine (LVII) and 95% H_2SO_4 as described in Example 20. Quantitative yield was isolated. 400 MHz ^{1}H-NMR (DMSO-d_6, ppm) 11.6-11.3 (1H, br s), 8.61-8.41 (0.8H, m), 8.18-8.03 (0.2H, m), 7.63-7.28 (1H, m), 4.14-4.08 (2H, m), 3.92-3.81 (2H, m, overlapped with water), 3.36-3.19 (4H, m), 1.86-1.78 (2H, m), 1.77-1.68 (2H, m), 1.6-1.45 (4H, m), 1.60-1.45 (6H, m). ESI-MS (m/z): 281 $[M+H]^+$. M.P.: 134-137° C.

Example 27

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isopropyl-N-methyl-hydroxylamine (LXIV)

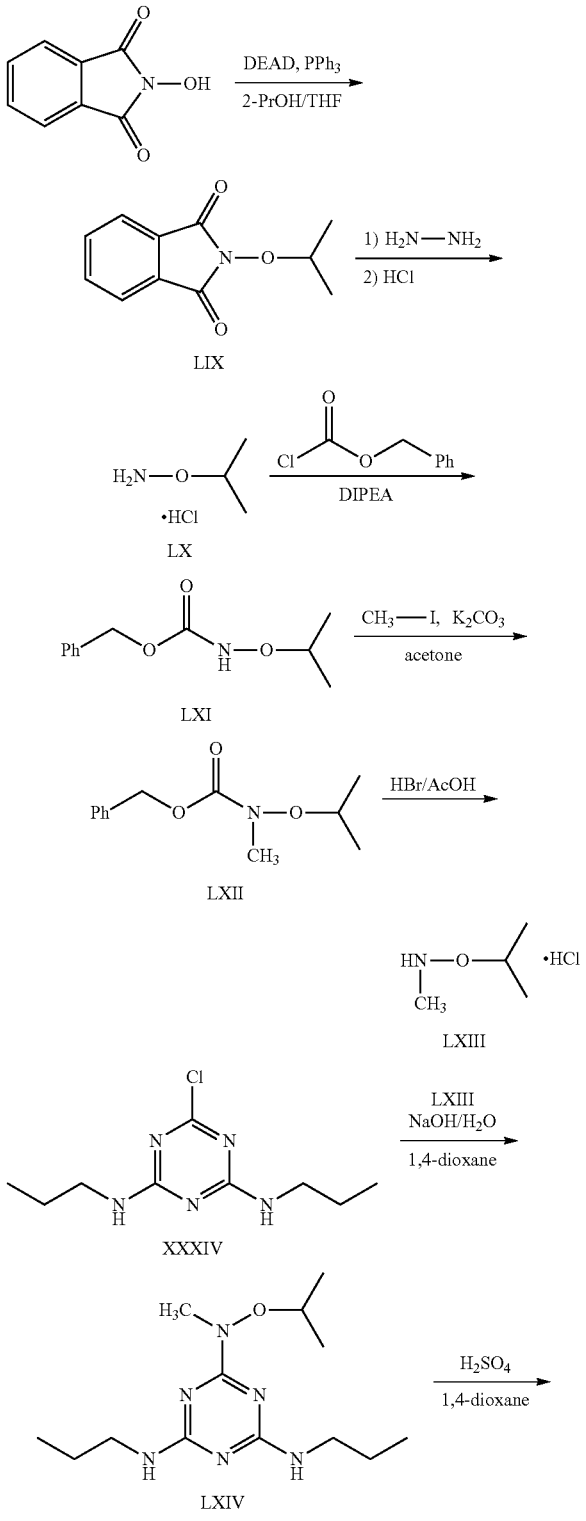
Example 28

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isopropyl-N-methyl-hydroxylamine hydrogen sulfate (LXV)

2-Isopropoxy-isoindole-1,3-dione (LIX)

Diethyl azodicarboxylate (14.5 mL, 73.56 mmol) was added dropwise at 0° C. to a stirred suspension of propan-2-ol (4.7 mL, 61.30 mmol), triphenylphosphine (19.30 g, 73.56 mmol), and N-hydroxyphthalimide (10.00 g, 61.30 mmol) in THF (50 mL). The mixture was stirred at room temperature for 20 h and evaporated to dryness. The product was purified by flash column chromatography using gradient elution from petroleum ether/EtOAc (9:1) to petroleum ether/EtOAc (5:1) to yield 2-isopropoxy-isoindole-1,3-dione (LIX, 10.92 g, 87%). 400 MHz ^{1}H NMR (DMSO-d_6, ppm): 7.86 (4H, s), 4.44 (1H, septet, J=6.2 Hz), 1.28 (6H, d, J=6.2 Hz).

Scheme 27



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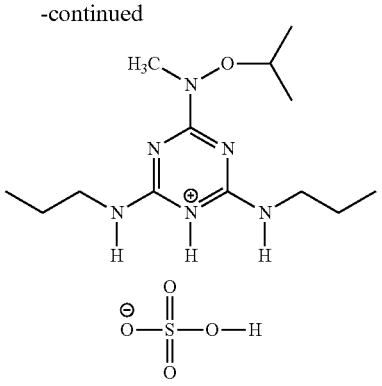


LXV

O-Isopropyl-hydroxylamine hydrochloride (LX)

A mixture of 2-isopropoxy-isoindole-1,3-dione (LIX, 10.78 g, 52.50 mmol) and hydrazine monohydrate (5.1 mL, 105.00 mmol) in CH_2Cl_2 (60 mL) was stirred at room temperature for 20 h. The reaction mixture was filtered. The filtrate was washed with water (70 mL), brine (70 mL) and dried over Na_2SO_4. After removing the drying agent via filtration, 4M HCl/1,4-dioxane (13.8 mL, 55.00 mmol) was added and the volatiles was removed under reduced pressure to yield O-isopropyl-hydroxylamine hydrochloride (LX, 3.91 g, 67% yield). 400 MHz ^{1}H NMR (DMSO-d_6, ppm) 11.04 (3H, br s), 4.35 (1H, septet, J=6.2 Hz), 1.21 (6H, d, J=6.2 Hz).

O-Benzyl-N-isopropoxy carbamate (LXI)

To a pre-cooled (0° C.) solution of O-isopropyl-hydroxylamine hydrochloride (3.89 g, 34.87 mmol) in CH_2Cl_2 (150 mL) was added N,N-diisopropylethylamine (14.4 mL, 87.18 mmol) and benzyl chloroformate (5.0 mL, 34.87 mmol). The resulting solution was stirred at room temperature for 5 h. At this time the solution was washed twice with saturated aqueous $NaHCO_3$ (30 mL) and dried over Na_2SO_4. The product was purified by flash column chromatography using gradient elution from petroleum ether/EtOAc (95:5) to petroleum ether/EtOAc (6:1) to yield O-benzyl-N-isopropoxycarbamate (LXI, 4.98 g, 68%). 400 MHz ^{1}H NMR (DMSO-d_6, ppm) 10.22 (1H, s), 7.42-7.29 (5H, m), 5.07 (2H, s), 3.89 (1H, septet, J=6.2 Hz), 1.11 (6H, d, J=6.2 Hz).

O-Benzyl-N-methyl-N-isopropoxy carbamate (LXII)

An ACE® pressure tube was charged with benzyl isopropoxycarbamate (4.98 g, 23.80 mmol), anhydrous K_2CO_3 (4.94 g, 35.70 mmol), methyl iodide (6.7 mL, 107.10), and anhydrous acetone (30 mL). The reaction mixture was heated at 70° C. for 24 h. The reaction mixture was filtered, and the acetone was evaporated. The resulting slurry was dissolved in EtOAc, washed with water (3×50 mL), dried (Na_2SO_4), and filtered. The solvent was removed to yield benzyl isopropoxy (methyl)carbamate (4.96 g, 93%). 400 MHz ^{1}H-NMR (DMSO-$_{d6}$, ppm) 7.41-7.30 (5H, m), 5.12 (2H, s), 4.08 (1H, septet, J=6.2 Hz), 3.08 (3H, s), 1.12 (6H, d, J=6.2 Hz).

O-Isopropyl-N-methyl-hydroxylamine hydrochloride (LXIII)

O-Benzyl-N-methyl-Nisopropoxy carbamate (4.96 g, 22.22 mmol) and 33% HBr/AcOH (45 mL) were stirred at room temperature for 20 min. Saturated solution of $NaHCO_3$ (400 mL) was added, the suspension was extracted with CH_2Cl_2 (3×150 mL). The combined organic extracts were dried over Na_2SO_4. After removal of the drying agent via filtration, 4M HCl/1,4-dioxane (6.7 mL, 26.65 mmol) was added, and the volatiles was removed under reduced pressure to yield O-isopropyl-N-methyl-hydroxylamine hydrochloride (2.09 g, 75%). 400 MHz ^{1}H NMR (DMSO-d_6, ppm) 12.3-11.7 (2H, br s), 4.49 (1H, septet, J=6.1 Hz), 2.77 (3H, s), 1.12 (6H, d, J=6.1 Hz).

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isopropyl-N-methyl-hydroxylamine (LXIV)

A mixture of 2-chloro-N-(4,6-bis-(n-propylamino)-[1,3,5] triazine (XXXIV) (1.65 g, 16.61 mmol), O-isopropyl-N-methyl-hydroxylamine hydrochloride (LXIII, 2.09 g, 16.61 mmol) and NaOH (0.66 g, 16.61 mmol) in 1,4-dioxane (50 mL) and water (5 mL) was heated at 100° C. for 16 h. The volatiles were then removed under reduced pressure. Saturated $NaHCO_3$ solution (50 mL) was added to the residue and the mixture was extracted with EtOAc (3×50 mL). The combined organic extracts were washed with water (50 mL), brine (50 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography using gradient elution from CH_2Cl_2/EtOH (99:1) to CH_2Cl_2/EtOH (95:5) to yield N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isopropyl-N-methyl-hydroxylamine (LXIV, 1.93 g, 95%). ESI-MS (m/z): 283 $[M+H]^+$.

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isopropyl-N-methyl-hydroxylamine hydrogen sulfate (LXV)

To a solution N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isopropyl-N-methyl-hydroxylamine (LXIV, 1.93 g, 6.83 mmol) in 1,4-dioxane (6 mL) at 0° C. was added 95% H_2SO_4 (0.36 mL, 6.83 mmol) in a drop-wise manner. The mixture was stirred for 0.5 h at room temperature and then the volatiles were removed under reduced pressure. The residue was co-evaporated with dry toluene (3×25 mL) to yield N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isopropyl-N-methyl-hydroxylamine hydrogen sulfate (~quantitative yield). 400 MHz ^{1}H-NMR (DMSO-d_6, ppm) 11.3-10.7 (1H, br s), 8.8-8.4 (1H, br s), 8.2-8.0 (0.3H, br s), 8.04-7.65 (0.7H, m), 4.43-4.28 (1H, m), 3.42-3.18 (7H, m), 1.64-1.44 (4H, m), 1.25 (6H, d, J=6.1 Hz), 0.94-0.82 (6H, m). ESI-MS (m/z): 283 $[M+H]^+$.

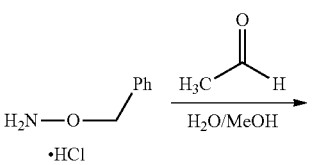
Example 29

O-Benzyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N-ethyl-hydroxylamine (LXVIII)

Example 30

O-Benzyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N-ethyl-hydroxylamine hydrogen sulfate (LXIX)

Scheme 28



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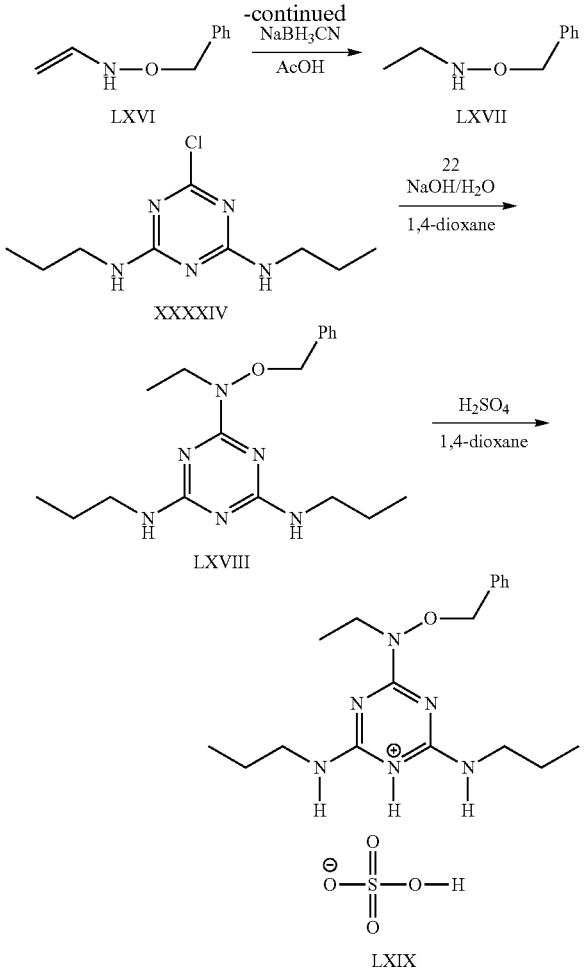


O-Benzyl-N-vinyl-hydroxylamine (LXVI)

Acetaldehyde (24.7 mL, 44.2 mmol) was added dropwise to the cooled solution (0° C.) of O-benzyl-hydroxylamine hydrochloride (7.00 g, 43.85 mmol) in water (100 mL) and MeOH (20 mL). The reaction mixture was stirred for 16 h. The volatiles were removed under reduced pressure, and the water suspension was extracted with EtOAc (2×75 mL). The combined organic extracts were washed with brine, and then dried over Na_2SO_4 and evaporated to yield O-benzyl-N-vinyl-hydroxylamine (LXVI) in quantitative yield. 400 MHz ^{1}H NMR (DMSO-d_6, ppm) 7.48 (0.5H, q, J=5.8 Hz), 7.39-7.26 (5H, m), 6.86 (0.5H, q, J=5.5 Hz), 5.06 (1H, s), 4.97 (1H, s), 1.78 (1.5H, d, J=5.5 Hz), 1.76 (1.5H, d, J=5.8 Hz).

O-Benzyl-N-ethyl-hydroxylamine (LXVII)

To a solution of O-benzyl-N-vinyl-hydroxylamine (LXVI, 6.52 g, 43.70 mmol) in AcOH (10 mL), $NaCNBH_3$ (11.00 g, 175.05 mmol) was added in portions. The reaction mixture was stirred at room temperature for 1 h. The mixture was neutralized (pH 7) with 1N NaOH and extracted with EtOAc (3×75 mL). The combined organic extracts were washed with saturated $NaHCO_3$ solution (2×100 mL), dried over Na_2SO_4, and purified by flash column chromatography (eluent: petroleum ether/EtOAc (9:1) to petroleum ether/EtOAc (1:4)) to yield O-benzyl-N-ethyl-hydroxylamine (LXVII, 2.10 g, 32%). 400 MHz ^{1}H NMR (DMSO-d_6, ppm) 7.36-7.24 (5H, m), 6.50 (1H, t, J=6.4 Hz), 4.60 (2H, s), 2.81 (2H, qd, J=7.0, 6.4 Hz), 0.98 (3H, t, J=7.0 Hz).

O-Benzyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N-ethyl-hydroxylamine (LXVIII)

2-Chloro-N-(4,6-bis-(n-propylamino)-[1,3,5]triazine (XXXIV) and O-benzyl-N-ethyl-hydroxylamine (LXVII) were reacted as described in Example 13 to afford O-benzyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N-ethyl-hydroxylamine (LXVIII, 38% yield). 400 MHz ^{1}H NMR (DMSO-d_6, ppm) 7.51-7.45 (2H, m), 7.40-7.30 (3H, s), 6.97-6.85 (1H, m), 6.79-6.67 (1H, m), 4.97-4.87 (1H, m), 3.72-3.53 (2H, m), 3.23-3.11 (4H, m), 1.56-1.43 (4H, m), 1.13-1.00 (3H, m), 0.89-0.80 (6H, m). ESI-MS (m/z): 345 $[M+H]^+$.

O-Benzyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N-ethyl-hydroxylamine hydrogen sulfate (LXIX)

O-Benzyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N-ethyl-hydroxylamine (LXVIII) was reacted with 95% H_2SO_4 as described in Example 9 to yield O-benzyl-N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N-methyl-hydroxylamine hydrogen sulfate (LXIX) in quantitative yield. 400 MHz ^{1}H NMR (DMSO-d_6, ppm) 12.0-11.0 (1H, br s), 8.7-8.0 (1H, m), 7.57-7.30 (5H, m), 5.07-4.95 (2H, m), 3.89-3.68 (2H, m), 3.39-3.14 (4H, m), 1.63-1.42 (4H, m), 1.23-1.07 (3H, m), 0.94-0.77 (6H, m). ESI-MS (m/z): 345 $[M+H]^+$.

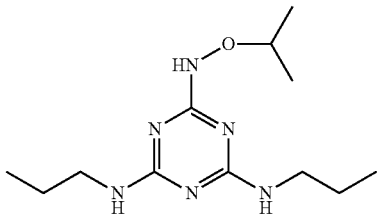
Example 31

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isopropyl-hydroxylamine (LXX)

Example 32

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isopropyl-hydroxylamine hydrogen sulfate (LXXI)

Scheme 29



N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isopropyl-hydroxylamine (LXX)

2-Chloro-N-(4,6-bis-(n-propylamino)-[1,3,5]triazine (XXXIV) and O-isopropyl-hydroxylamine hydrochloride were reacted as described in Example 13 (80% yield). ESI-MS (m/z): 269 $[M+H]^+$.

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isopropyl-hydroxylamine hydrogen sulfate (LXXI)

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isopropyl-hydroxylamine (LXX) was reacted with 95% H_2SO_4 as

described in Example 20 (quantitative yield). 400 MHz ^{1}H NMR (DMSO-d_6, ppm) 11.5-10.7 (1H, m), 8.6-7.5 (3H, m), 4.08 (1H, septet, J=6.2 Hz), 3.38-3.13 (4H, m), 1.61-1.44 (4H, m), 1.21 (6H, d, J=6.2 Hz), 0.94-0.81 (6H, m). ESI-MS (m/z): 269 $[M+H]^+$.

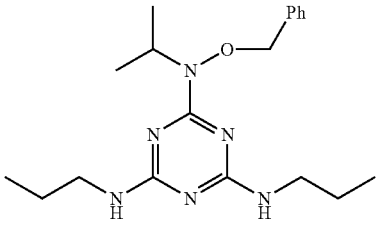
Example 33

6-((Benzyloxy)(isopropyl)amino)-N^2,N^4-dipropyl-1,3,5-triazine-2,4-diamine (LXXII)

Example 34

6-((Benzyloxy)(isopropyl)amino)-N^2,N^4-dipropyl-1,3,5-triazine-2,4-diamine hydrogen sulfate (LXXIII)

Scheme 30



LXXII

6-((Benzyloxy)(isopropyl)amino)-N^2,N^4-dipropyl-1,3,5-triazine-2,4-diamine (LXXII) was prepared by reacting 2-chloro-N-(4,6-bis-(n-propylamino)-[1,3,5]triazine (XXXIV) and O-benzyl-N-isopropyl-hydroxylamine as exemplified in Example 13. The corresponding hydrogen sulfate (LXXIII) was prepared as described in Example 20.

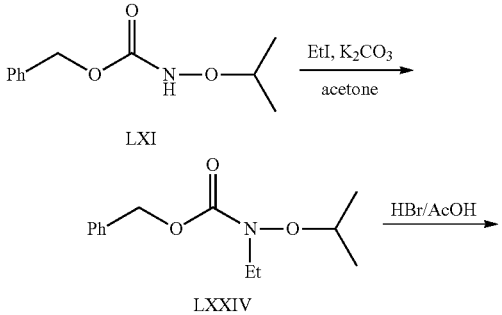
Example 35

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N-ethyl-O-isopropyl-hydroxylamine (LXXVI)

Example 36

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N-ethyl-O-isopropyl-hydroxylamine hydrogen sulfate (LXXVII)

Scheme 31



LXI

LXXIV

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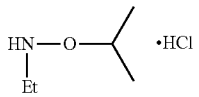
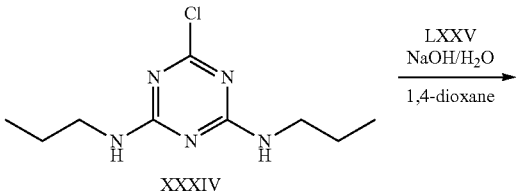
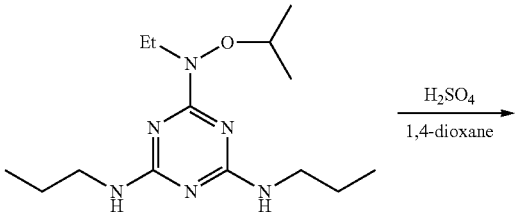


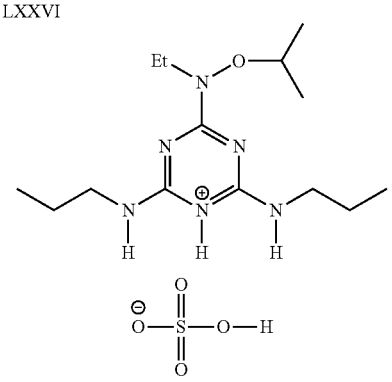
LXXV



XXXIV



LXXVI



LXXVII

O-Benzyl-N-ethyl-N-isopropoxy-carbamate (LXXIV)

An ACE® pressure tube was charged with benzyl isopropoxycarbamate (4.08 g, 19.50 mmol), anhydrous K_2CO_3 (4.04 g, 29.25 mmol), ethyl iodide (7.0 mL, 87.75 mmol), and anhydrous acetone (30 mL). The reaction mixture was heated at 70° C. for 24 h. Ethyl iodide (7.0 mL, 87.75 mmol) and K_2CO_3 (4.04 g, 29.25 mmol) were added and the reaction mixture was heated for 24 h. The reaction mixture was filtered, and the acetone was evaporated. The resulting slurry was dissolved in EtOAc (150 mL), washed with water (3×50 mL), dried (Na_2SO_4), and filtered. The solvent was removed to yield O-benzyl-N-ethyl-N-isopropoxy-carbamate (3.86 g, 83%). 400 MHz ^{1}H NMR (DMSO-d_6, ppm) 7.41-7.30 (5H, m), 5.12 (2H, s), 4.05 (1H, septet, J=6.2 Hz), 3.46 (2H, q, J=7.0 Hz), 1.12 (6H, d, J=6.2 Hz), 1.06 (3H, t, J=7.0 Hz).

N-Ethyl-O-isopropyl-hydroxylamine hydrochloride (LXXV)

O-Benzyl-N-ethyl-N-isopropoxy-carbamate was reacted with HBr/AcOH as described for the preparation of compound LXIII in Example 27 (yield 71%). 400 MHz ^{1}H NMR (DMSO-d_6, ppm) 11.7-11.2 (2H, br s), 4.41 (1H, septet, J=6.1 Hz), 3.16 (2H, q, J=7.2 Hz), 1.24 (6H, d, J=6.1 Hz), 1.19 (3H, t, J=7.2 Hz).

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N-ethyl-O-isopropyl-hydroxylamine (LXXVI)

2-Chloro-N-(4,6-bis-(n-propylamino)-[1,3,5]triazine (XXXIV) was reacted with N-ethyl-O-isopropyl-hydroxylamine hydrochloride (LXXV) as described in Example 13, yielding N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N-ethyl-O-isopropyl-hydroxylamine (LXXVI) (88% yield). ESI-MS (m/z): 297 $[M+H]^+$.

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N-ethyl-O-isopropyl-hydroxylamine hydrogen sulfate (LXXVII)

N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N-ethyl-O-isopropyl-hydroxylamine (LXXVI) was reacted with 95% H_2SO_4 as described in Example 20 (quantitative yield). 400 MHz ^1H NMR (DMSO-d_6, ppm) 12.0-10.8 (1H, m), 8.7-8.4 (1H, br s), 8.27-7.78 (1H, m), 4.39-4.25 (1H, m), 3.90-3.76 (2H, m), 3.39-3.15 (4H, m), 1.62-1.45 (4H, m), 1.25 (6H, d, J=6.1 Hz), 1.18-1.10 (3H, m), 0.95-0.82 (6H, m). ESI-MS (m/z): 297 $[M+H]^+$.

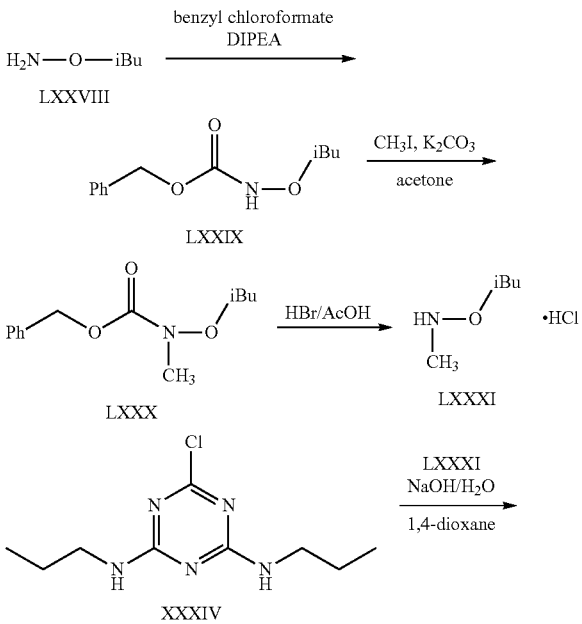
Example 37

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isobutyl-N-methyl-hydroxylamine (LXXXII)

Example 38

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isobutyl-N-methyl-hydroxylamine hydrogen sulfate (LXXXIII)

Scheme 32



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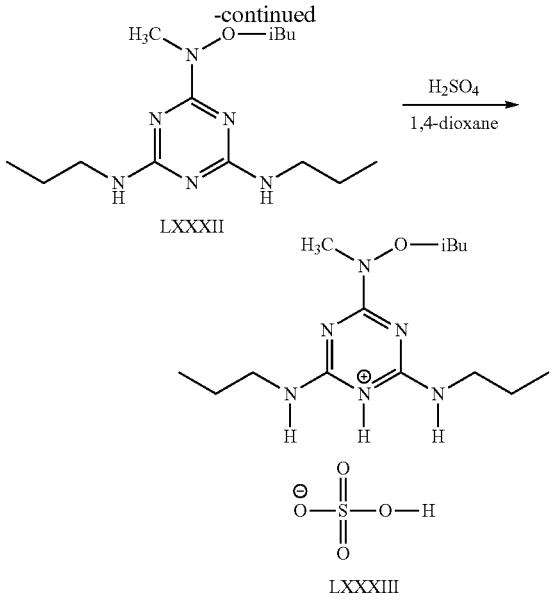


O-Benzyl-N-isobutoxy carbamate (LXXIX)

O-isobutyl-hydroxylamine hydrochloride (LXXVIII) was reacted with benzyl chloroformate as described for the preparation of compound LXI in Example 27, yielding O-benzyl-N-isobutoxy carbamate (LXXIX) (87% yield). 400 MHz ^1H NMR ($CDCl_3$, ppm) 7.36-7.23 (5H, m), 5.11 (2H, s), 3.58 (2H, d, J=6.6 Hz), 1.89 (1H, septet, J=6.7 Hz), 0.86 (6H, d, J=6.7 Hz).

O-Benzyl-N-methyl-N-isobutoxy carbamate (LXXX)

O-Benzyl-N-isobutoxycarbamate was reacted with methyl iodide, as described for the preparation of compound LXII in Example 27, affording O-benzyl-N-methyl-N-isobutoxycarbamate in 78% yield. 400 MHz ^1H-NMR (DMSO-d_6, ppm) 7.41-7.30 (5H, m), 5.12 (2H, s), 3.59 (2H, d, J=6.6 Hz), 3.09 (3H, s), 1.80 (1H, septet, J=6.7 Hz), 0.87 (6H, d, J=6.7 Hz).

O-Isobutyl-N-methyl-hydroxylamine hydrochloride (LXXXI)

O-Benzyl-N-methyl-N-isobutoxycarbamate was reacted with HBr/AcOH as described for the preparation of compound LXIII in Example 27 (38% yield). 200 MHz ^1H NMR (DMSO-d_6, ppm) 12.5-11.4 (2H, br s), 3.84 (2H, d, J=6.6 Hz), 2.81 (3H, s), 1.90 (1H, septet, J=6.7 Hz), 0.89 (6H, d, J=6.7 Hz).

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isobutyl-N-methyl-hydroxylamine (LXXXII)

2-Chloro-N-(4,6-bis-(n-propylamino)-[1,3,5]triazine (XXXIV) was reacted with O-isobutyl-N-methyl-hydroxylamine hydrochloride as described in Example 13, affording LXXXII in 82% yield. ESI-MS (m/z) 297 $[M+H]^+$.

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isobutyl-N-methyl-hydroxylamine hydrogen sulfate (LXXXIII)

N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-O-isobutyl-N-methyl-hydroxylamine (LXXXII) was reacted with

95% H_2SO_4 as described in Example 20 (quantitative yield). 400 MHz ^{1}H-NMR (DMSO-d_6, ppm) 12.0-10.7 (1H, br s), 8.7-7.6 (2H, m), 3.82-3.72 (2H, m), 3.41-3.20 (7H, m), 2.11-1.82 (1H, m), 1.62-1.44 (4H, m), 1.00-0.82 (12H, m). ESI-MS (m/z): 297 $[M+H]^+$.

Example 39

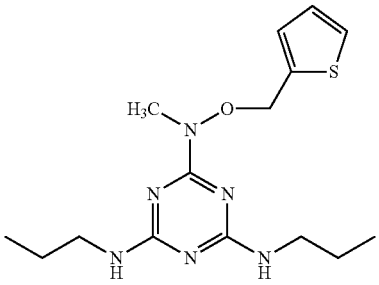
6-(Methyl(thiophen-2-ylmethoxy)amino)-N^2,N^4-di-n-propyl-1,3,5-triazine-2,4-diamine (LXXXIV)

Example 40

6-(Methyl(thiophen-2-ylmethoxy)amino)-N^2,N^4-di-n-propyl-1,3,5-triazine-2,4-diamine hydrogen sulfate (LXXXV)

Scheme 33

LXXXIV



6-(Methyl(thiophen-2-ylmethoxy)amino)-N^2,N^4-di-n-propyl-1,3,5-triazine-2,4-diamine (LXXXIV) may be prepared by reacting 2-chloro-N-(4,6-bis-(n-propylamino)-[1,3,5]triazine (XXXIV) and O-(thiophen-2-yl-methyl)-N-methyl-hydroxylamine as exemplified in Example 13.

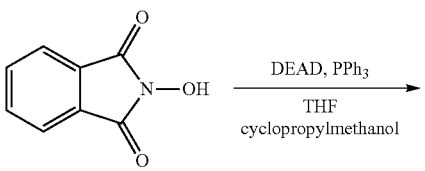
Example 41

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-cyclopropylmethyl-N-methyl-hydroxylamine (XCI)

Example 42

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-cyclopropylmethyl-N-methyl-hydroxylamine hydrogen sulfate (XCII)

Scheme 34



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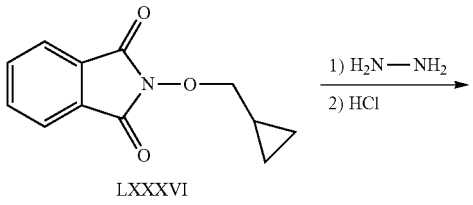
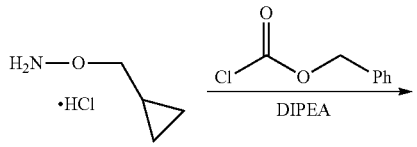
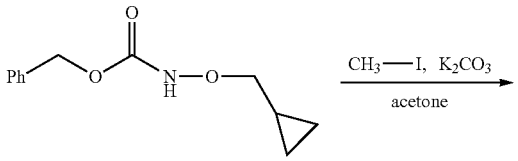


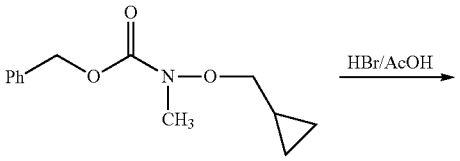
LXXXVI



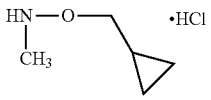
LXXXVII



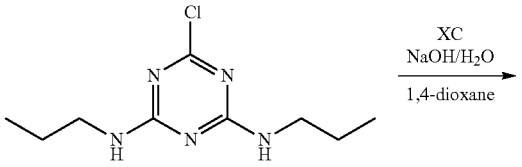
LXXXVIII



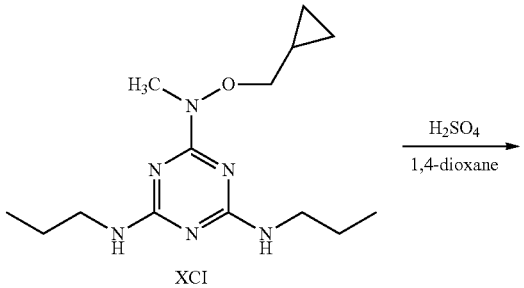
LXXXIX



XC



XXXIV



XCI

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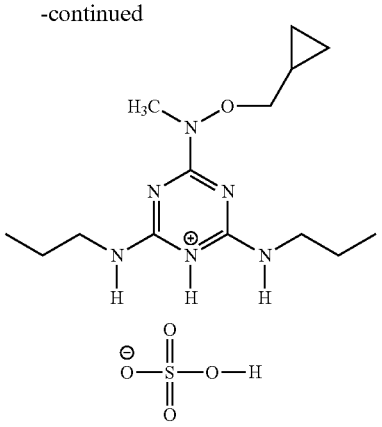


XCII

2-Cyclopropylmethoxy-isoindole-1,3-dione (LXXXVI)

N-Hydroxyphthalimide and cyclopropyl-methanol were reacted as described for compound LIX in Example 27, to afford LXXXVI in 87% yield. 400 MHz ^{1} NMR (DMSO-d_6, ppm) 7.86 (4H, s), 3.97 (2H, d, J=7.4 Hz), 1.22-1.11 (1H, m), 0.61-0.48 (2H, m), 0.34-0.22 (2H, m).

O-Cyclopropylmethyl-hydroxylamine hydrochloride (LXXXVII)

2-Cyclopropylmethoxy-isoindole-1,3-dione was reacted with hydrazine as described for compound LX in Example 27 (LXXXVII, in 67% yield). 400 MHz ^{1}H NMR (DMSO-d_6, ppm) 10.95 (3H, br s), 3.83 (2H, d, J=7.4 Hz), 1.12-1.01 (1H, m), 0.62-0.50 (2H, m), 0.35-0.24 (2H, m).

O-Benzyl-N-cyclopropylmethoxy carbamate (LXXXVIII)

O-Cyclopropylmethyl-hydroxylamine hydrochloride was reacted with benzyl chloroformate as described for compound LXI in Example 27 (LXXXVIII, in 88% yield). 400 MHz ^{1}H-NMR (DMSO-d_6, ppm) 10.37 (1H, br s), 7.41-7.30 (5H, m), 5.08 (2H, s), 3.54 (2H, d, J=7.2 Hz), 1.06-0.92 (1H, m), 0.54-0.41 (2H, m), 0.25-0.13 (2H, m).

O-Benzyl-N-methyl-N-cyclopropylmethoxy-carbamate (LXXXIX)

O-Benzyl N-cyclopropylmethoxy carbamate and methyl iodide were reacted as described for compound LXII in Example 27 (LXXXIX, in 95% yield). 400 MHz ^{1}H NMR (DMSO-d_6, ppm) 7.42-7.30 (5H, m), 5.11 (2H, s), 3.62 (2H, d, J=7.2 Hz), 3.11 (3H, s), 1.06-0.93 (1H, m), 0.54-0.41 (2H, m), 0.26-0.13 (2H, m).

O-Cyclopropylmethyl-N-methyl-hydroxylamine hydrochloride (XC)

O-Benzyl-N-methyl-N-cyclopropylmethoxy carbamate was reacted with HBr/AcOH as described for compound LXIII in Example 27, yielding XC in 77% yield. 400 MHz ^{1}H NMR (DMSO-d_6, ppm) 11.96 (2H, br s), 3.91 (2H, d, J=7.4 Hz), 2.80 (3H, s), 1.13-1.01 (1H, m), 0.63-0.50 (2H, m), 0.37-0.25 (2H, m).

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-cyclopropylmethyl-N-methyl-hydroxylamine (XCI)

2-Chloro-N-(4,6-bis-(n-propylamino)-[1,3,5]triazine (XXXIV) was reacted with O-cyclopropylmethyl-N-methyl-hydroxylamine hydrochloride (XC) as described in Example 13, yielding (XCI) in 99% yield. 400 MHz ^{1}H NMR (DMSO-d_6, ppm) 6.91-6.77 (1H, m), 6.75-6.58 (1H, m), 3.77-3.64 (2H, m), 3.21-3.09 (7H, m), 1.54-1.41 (4H, m), 1.11-1.00 (1H, m), 0.88-0.80 (6H, m), 0.56-0.44 (2H, m), 0.32-0.20 (2H, m). ESI-MS (m/z) 295 $[M+H]^+$.

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-cyclopropylmethyl-N-methyl-hydroxylamine hydrogen sulfate (XCII)

N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-O-cyclopropylmethyl-N-methyl-hydroxylamine (XCI) was reacted with 95% H_2SO_4 as described in Example 20, yielding (XCII) in quantitative yield. 400 MHz ^{1}H-NMR (DMSO-d_6, ppm) 11.6-11.0 (1H, br s), 8.7-8.4 (0.7H, br s), 8.2-8.0 (0.3H, br s), 7.89-7.42 (1H, m), 3.88-3.77 (2H, m), 3.42-3.18 (7H, m), 1.62-1.45 (4H, m), 1.24-1.13 (1H, m), 0.95-0.82 (6H, m), 0.61-0.52 (2H, m), 0.38-0.28 (2H, m). ESI-MS (m/z) 295 $[M+H]^+$.

Example 43

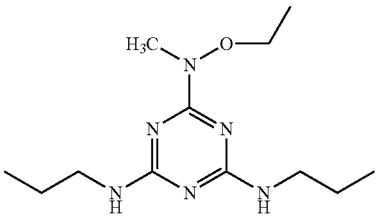
N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-ethyl-N-methyl-hydroxylamine (XCVI)

Example 44

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-ethyl-N-methyl-hydroxylamine hydrogen sulfate (XCVII)

Scheme 35

XCVI



tert-Butyl ethoxycarbamate (XCIII)

A solution of saturated Na_2CO_3 solution (45.0 mL) was added dropwise at room temperature to a solution of O-ethyl-hydroxylamine (1.76 g, 18.0 mmol) and di-tert-butyl dicarbonate (5.13 g, 23.67 mmol) in dichloromethane (45.0 mL) and stirred for 24 h. Water was added, the mixture pH was adjusted to 2 by adding 6N HCl, and the resulting system was extracted with dichloromethane (3×50 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated. The crude product was purified by flash column chromatography using gradient elution from petroleum ether/EtOAc (98:2) to petroleum ether/EtOAc (95:5) to yield tert-butyl ethoxycarbamate (XCIII) (2.62 g, 90%). 400 MHz ^{1}H NMR (DMSO-d_6, ppm) 9.89 (1H, s) 3.72 (2H, q, J=7.0 Hz) 1.40 (9H, s) 1.10 (3H, t, J=7.0 Hz).

tert-Butyl ethoxy(methyl)carbamate (XCIV)

A solution of tert-butyl ethoxycarbamate (XCIII, 2.48 g, 15.38 mmol) in DMF (10 mL) was added dropwise to the

suspension of 60% sodium hydride (0.66 g, 16.92 mmol) in DMF (5 mL) at 0° C. After 30 min. methyl iodide (1.95 mL, 31.32 mmol) in DMF (10 mL) was added, and the reaction mixture was stirred at room temperature for 24 h. Water (100 mL) was added and the product was extracted with ethyl acetate (3×50 mL), dried over Na_2SO_4, and concentrated in vacuo to yield tert-butyl ethoxy(methyl)carbamate (XCIV, 2.34 g, 87%). 400 MHz ^{1}H NMR (DMSO-d_6, ppm) 3.81 (2H, q, J=7.0 Hz), 3.00 (3H, s), 1.41 (9H, s), 1.12 (3H, t, J=7.0 Hz).

O-Ethyl-N-methyl-hydroxylamine hydrochloride (XCV)

A solution of 4M HCl/1,4-dioxane (25 mL) was added in portions to tert-butyl ethoxy(methyl)carbamate (XCIV, 2.34 g, 13.35 mmol) at 0° C. The mixture was stirred at room temperature for 4 h. The volatiles removed in vacuo and the residue was triturated with ethyl ether and filtered to yield a solid (O-ethyl-N-methyl-hydroxylamine hydrochloride, XCV, 1.35 g, 91%).

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-ethyl-N-methyl-hydroxylamine (XCVI)

2-Chloro-N-(4,6-bis-(n-propylamino)-[1,3,5]triazine (XXXIV) was reacted with O-ethyl-N-methyl-hydroxylamine hydrochloride as described in Example 13, to yield XCVI in 93% yield. ESI-MS (m/z) 269 $[M+H]^+$.

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-ethyl-N-methyl-hydroxylamine hydrogen sulfate (XCVII)

N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-O-ethyl-N-methyl-hydroxylamine (XCVI) was reacted with 95% H_2SO_4 as described in Example 20, to afford (XCVII) in 91% yield. 400 MHz ^{1}H NMR (DMSO-d_6, ppm) 11.7-10.8 (1H, br s), 8.79-7.34 (2H, m), 4.09-3.98 (2H, m), 3.40-3.20 (7H, m), 1.61-1.46 9 (4H, m), 1.27 (3H, t, J=7.1 Hz), 0.94-0.84 (6H, m). 400 MHz ^{1}HNMR (D_2O, ppm) 3.99-3.88 (2H, m), 3.34-3.13 (7H, m), 1.52-1.39 (4H, m), 1.14 (3H, t, J=7.1 Hz), 0.76 (6H, t, J=7.5 Hz). ESI-MS (m/z): 269 $[M+H]^+$. M.P.: 84-86° C.

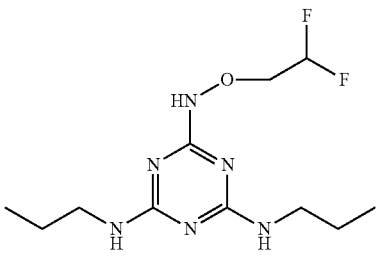
Example 45

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-(2,2-difluoro-ethyl)-hydroxylamine (C)

Example 46

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-(2,2-difluoro-ethyl)-hydroxylamine hydrogen sulfate (CI)

Scheme 36



C

2-(2,2-Difluoro-ethoxy)-isoindole-1,3-dione (XCVIII)

N-Hydroxyphthalimide and 2,2-difluoro-ethanol were reacted as described for compound LXI in Example 27, affording XCVIII in 52% yield. 400 MHz ^{1}H NMR (DMSO-d_6, ppm) 7.92-7.85 (4H, m), 6.34 (1H, tt, J=54.4, 3.9 Hz), 4.46 (2H, td, J=14.1, 3.9 Hz).

O-(2,2-Difluoro-ethyl)-hydroxylamine hydrochloride (XCIX)

2-(2,2-Difluoro-ethoxy)-isoindole-1,3-dione was reacted with hydrazine as described for compound LX in Example 27, affording XCIX in 73% yield. 400 MHz ^{1}H NMR (DMSO-d_6, ppm) 12.3-10.3 (3H, br s), 6.38 (1H, tt, J=54.0, 3.3 Hz), 4.34 (2H, td, J=14.7, 3.3 Hz).

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-(2,2-difluoro-ethyl)-hydroxylamine (C)

2-Chloro-N-(4,6-bis-(n-propylamino)-[1,3,5]triazine (XXXIV) was reacted with O-(2,2-difluoro-ethyl)-hydroxylamine hydrochloride as described in Example 13, affording C in 59% yield. 400 MHz ^{1}H NMR (DMSO-d_6, ppm) 9.91-9.56 (1H, m), 7.00-6.90 (1H, m), 6.89-6.67 (1H, m), 6.48-6.13 (1H, m, 4.12-3.98 (2H, m), 3.20-3.09 (4H, m), 1.53-1.41 (4H, m), 0.88-0.80 (6H, m). ESI-MS (m/z) 291 $[M+H]^+$.

N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-O-(2,2-difluoro-ethyl)-hydroxylamine hydrogen sulfate (CI)

N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-O-(2,2-difluoro-ethyl)-hydroxylamine (C, 1.02 g, 3.51 mmol) was reacted with 95% H_2SO_4 (0.19 mL, 3.51 mmol) in diethyl ether (3 mL) at 0° C. Two drops of EtOH were added, and the resultant crystals were filtered, washed with diethyl ether, and dried to yield N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-O-(2,2-difluoro-ethyl)-hydroxylamine hydrogen sulfate (CI) (1.26 g, 93%). 400 MHz ^{1}H NMR (DMSO-d_6, ppm) 11.8-10.5 (1H, m) 8.8-8.4 (0.3H, br s) 8.36-7.53 (1.7H, m) 6.50-6.08 (1H, m) 4.28-4.07 (2H, m) 3.39-3.13 (4H, m) 1.64-1.42 (4H, m) 0.97-0.78 (6H, m). ESI-MS (m/z) 291 $[M+H]^+$. M.P.: 91-93° C.

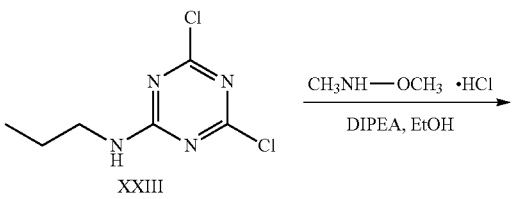
Example 47

4-N-(2-Dimethylaminoethyl)amino-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CIII)

Example 48

4-N-(2-Dimethylaminoethyl)amino-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (CIV)

Scheme 37



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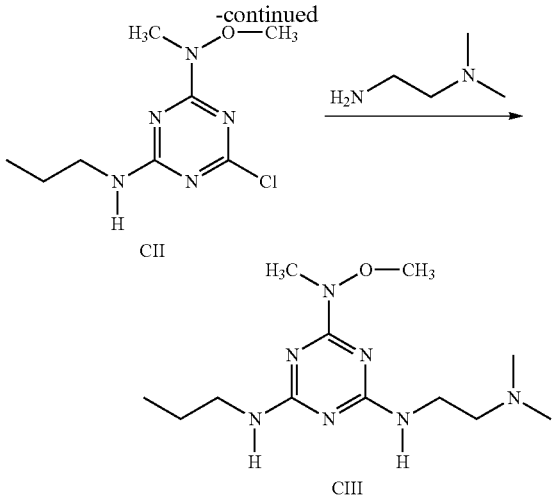


CII

CIII

2-Chloro-6-N-(n-propyl)aminol-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CII)

2,4-Dichloro-N-(6-n-propylamino)-[1,3,5]triazine (XXIII) (18 g, 87 mmol) was dissolved in acetone (100 mL) and poured into ice-water (50 mL) to form a very fine suspension. A solution of N,O-dimethylhydroxylamine hydrochloride (9.3 g, 95 mmol) in water (30 mL) was added, while keeping the temperature at 0° C. (ice bath). To this mixture, 2N NaOH (44 mL, 88 mmol) was added dropwise at a rate adjusted to keep the temperature between 0° C. and 5° C. The reaction was stirred for 30 min at ambient temperature and for additional 60 min at 50° C. The resultant precipitate was filtered off, and washed with water (3×25 mL). After drying over calcium chloride under high vacuum, 2-chloro-N-(6-n-propylamino)-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CII, 12 g, 60%) was isolated as a white powder. LCMS (ESI) m/z=232 $(M+H)^+$.

4-N-(2-Dimethylaminoethyl)amino-6-N-(n-propyl) amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CIII)

A mixture of 2-chloro-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CII, 1.5 g, 6.5 mmol), N,N-dimethylethane-1,2-diamine (3.5 g, 39 mmol) and DIPEA (2.5 g, 20 mmol) in EtOH (30 mL) was heated at 100° C. for 16 h. The solvent was then removed under reduced pressure. The residue was dissolved in EtOAc (80 mL), washed with water (2×50 mL) and then with a brine solution (50 mL) and lastly, dried over Na_2SO_4. The solvent was removed under reduced pressure. The residue was purified by flash column chromatography (DCM/MeOH=20/1 to 5/1) to yield 4-N-(2-dimethylaminoethyl)amino-6-N-(n-propyl) amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (620 mg, 33%).

4-N-(2-Dimethylaminoethyl)amino-6-N-(n-propyl) amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (CIV)

4-N-(2-dimethylaminoethyl)amino-6-N-(n-propyl) amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (610 mg, 2.1 mmol) was dissolved in H_2O (10 mL) and 0.5 M aqueous HCl solution (6.6 mL) and the solution was lyophilized to yield 4-N-(2-dimethylaminoethyl)amino-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (CIV, 630 mg) as a colorless oil. LCMS (ESI) m/z=284 $(M+H)^+$. ^1H NMR (500 MHz, DMSO) δ (ppm) 10.55-10.88 (br, 1H), 8.70-9.10 (m, 2H), 4.37-4.43 (m, 5H), 3.76-3.87 (m, 7H), 2.84-2.88 (m, 6H), 1.60-1.64 (m, 2H), 0.94-1.02 (m, 3H).

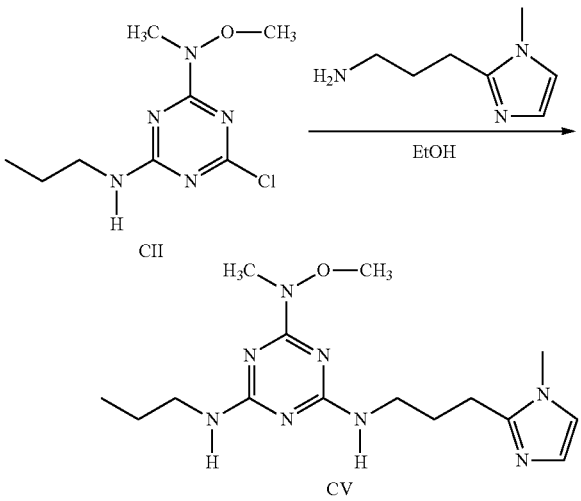
Example 49

4-N-(3-(1-N-Methylimidazol-2-yl)-propyl)-amino-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CV)

Example 50

4-N-(3-(1-N-Methylimidazol-2-yl)-propyl)-amino-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (CVI)

Scheme 38



CII

CV

4 N (3 (1-N-Methylimidazol-2-yl)-propyl)-amino-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CV)

2-Chloro-6-N-(n-propylamino)-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CII) (400 mg, 2.9 mmol), DIPEA (5.16 g, 40 mmol) and 3-(1-methyl-1H-imidazol-2-yl)propan-1-amine (J. Heterocyclic Chem., 2005, 42:1011-15) (732 mg, 3.2 mmol) in EtOH (50 mL) were heated at 100° C. for 16 h. After this time, the solvent was removed under reduced pressure. The residue was dissolved in DCM/MeOH (400 mL/200 mL), washed with water (50 mL) then dried over Na_2SO_4. The solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (DCM/MeOH=50/1 to 10/1) to yield 4-N-(3-(1-N-methylimidazol-2-yl)-propyl)-amino-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CV, 210 mg, 22%).

4 N (3 (1-N-Methylimidazol-2-yl)-propyl)-amino-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (CVI)

4-N-(3-(1-N-methylimidazol-2-yl)-propyl)-amino-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CV) (210 mg, 0.63 mmol) was dissolved in

H_2O (10 mL) and 0.5 M aqueous HCl solution (1.3 mL), and the solution was lyophilized to yield 4-N-(3-(1-N-methylimidazol-2-yl)-propyl)-amino-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (CVI, 210 mg) as a yellow oil. LCMS (ESI) m/z=335 $(M+H)^+$. ^1H NMR (500 MHz, DMSO) δ (ppm) 14.40-14.55 (br, 1H), 8.60-8.80 (m, 2H), 7.57-7.62 (m, 2H), 3.77-3.83 (m, 6H), 3.28-3.42 (m, 7H), 2.80-2.84 (m, 2H), 1.95-2.10 (m, 2H), 1.53-1.56 (m, 2H), 0.89-0.93 (m, 3H).

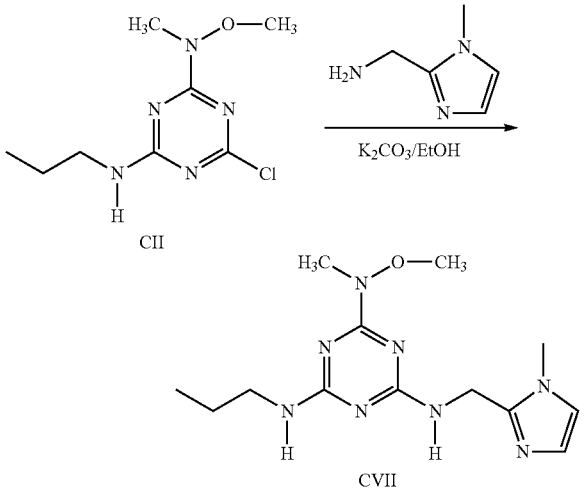
Example 51

4-N-(1-N-Methylimidazol-2-yl)-methylamino-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CVII)

Example 52

4-N-(1-N-Methylimidazol-2-yl)-methylamino-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (CVIII)

Scheme 39



4-N-(1-N-Methylimidazol-2-yl)-methylamino-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CVII)

2-Chloro-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CII) (1 g, 4.5 mmol), (1-methyl-1H-imidazol-2-yl)methanamine (600 mg, 5.4 mmol) and K_2CO_3 (1.24 g, 9 mmol) in EtOH (50 mL) were heated at 100° C. for 16 h. The reaction mixture was filtered, and the volatiles were removed under reduced pressure. The residue was dissolved in EtOAc (100 mL), washed with water (30 mL) and then with a brine solution (30 mL), and lastly dried over Na_2SO_4. The solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (DCM/MeOH=20/1 to 8/1) to yield 4-N-(1-N-methylimidazol-2-yl)-methylamino-6-N-(n-propyl)amino-[1,3,5] triazin-2-yl)-N,O-dimethyl-hydroxylamine (CVII, 650 mg, 47%).

4-N-(1-N-Methylimidazol-2-yl)-methylamino-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (CVIII)

4-N-(1-N-methylimidazol-2-yl)-methylamino-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CVII, 650 mg, 2.1 mmol) was dissolved in H_2O (10 mL) and 0.5 M aqueous HCl solution (6.3 mL). The resultant solution was subjected to lyophilization to yield 4-N-(1-N-methylimidazol-2-yl)-methylamino-6-N-(n-propyl)amino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (CVIII, 389 mg) as a colorless oil. LCMS (ESI) m/z=307 $(M+H)^+$. ^1H NMR (500 MHz, DMSO) δ (ppm) 14.65-14.85 (br, 1H), 8.70-9.20 (m, 2H), 7.62-7.70 (m, 2H), 4.87-4.91 (m, 2H), 3.75-3.89 (m, 9H), 3.31-3.40 (m, 3H), 1.55-1.56 (m, 2H), 0.85-0.96 (m, 3H).

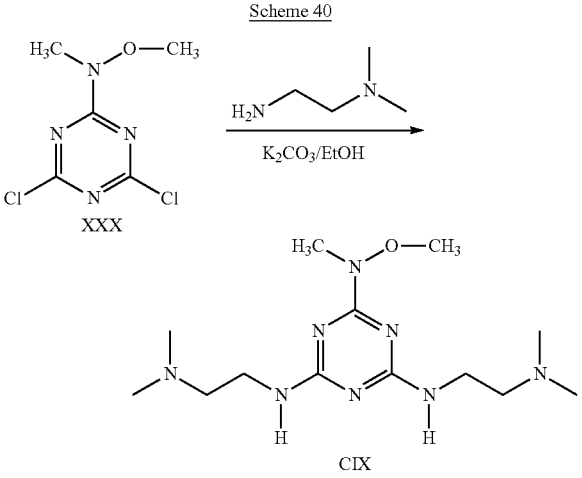
Example 53

4,6-Bis-(N-(2-dimethylaminoethyl)amino)-[1,3,5] triazin-2-yl)-N,O-dimethyl-hydroxylamine (CIX)

Example 54

4,6-Bis-(N-(2-dimethylaminoethyl)amino)-[1,3,5] triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (CX)

Scheme 40



4,6-Bis-(N-(2-dimethylaminoethyl)amino)-[1,3,5] triazin-2-yl)-N,O-dimethyl-hydroxylamine (CIX)

N-(4,6-Dichloro[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXX) (7 g, 33.5 mmol), N,N-dimethyl-ethane-1,2-diamine (6.05 g, 68.7 mmol) and K_2CO_3 (10.2 g, 73.7 mmol) in THF (250 mL) were heated at 70° C. for 5 h, after which time the solvent was removed under reduced pressure. The residue was purified by reverse flash column chromatography to yield 4,6-bis-(N-(2-dimethylaminoethyl)amino)-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (270 mg, 3%).

4,6-Bis-(N-(2-dimethylaminoethyl)amino)-[1,3,5] triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (CX)

4,6-Bis-(N-(2-dimethylaminoethyl)amino)-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (270 mg, 0.86 mmol)

was dissolved in H_2O (10 mL) and 0.5 M aqueous HCl solution (2 mL), and the resultant solution was lyophilized to yield 4,6-bis-(N-(2-dimethylaminoethyl)amino)-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (290 mg) as a colorless oil. LCMS (ESI) m/z=313 $(M+H)^+$. ^1H NMR (500 MHz, DMSO) δ (ppm) 10.20-10.60 (br, 1H), 7.17-7.25 (m, 2H), 3.54-3.76 (m, 7H), 3.01-3.25 (m, 7H), 2.72 (s, 12H).

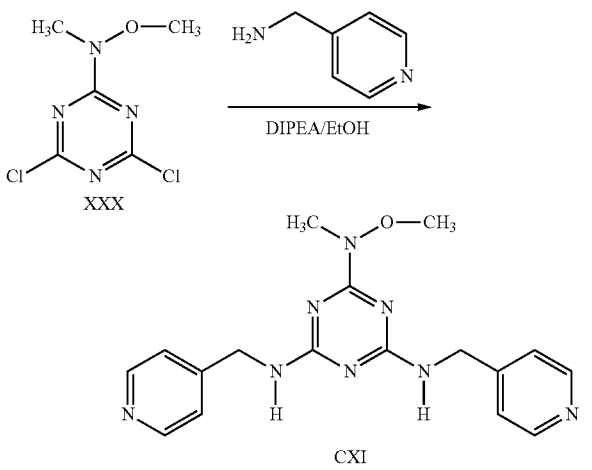
Example 55

4,6-Bis-(N-(pyridin-4-ylmethyl)amino)-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CXI)

Example 56

4,6-Bis-(N-(pyridin-4-ylmethyl)amino)-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (CXII)

Scheme 41



4,6-Bis-(N-(pyridin-4-ylmethyl)amino)-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CXI)

N-(4,6-Dichloro[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXX) (1 g, 4.78 mmol), pyridin-4-ylmethanamine (1.14, 10.52 mmol) and DIPEA (1.85 g, 14.34 mmol) in EtOH (80 mL) were heated at 100° C. for 16 h, after which the solvent was removed under reduced pressure. The crude product was purified by flash chromatography to yield 4,6-bis-(N-(pyridin-4-ylmethyl)amino)-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CXI) (450 mg, 27%).

4,6-Bis-(N-(pyridin-4-ylmethyl)amino)-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (CXII)

4,6-Bis-(N-(pyridin-4-ylmethyl)amino)-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CXI) (450 mg, 1.28 mmol) was dissolved in H_2O (10 mL) and 0.5 M aqueous HCl solution (3.84 mL), and the resultant solution was lyophilized to yield 4,6-bis-(N-(pyridin-4-ylmethyl)amino)-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (497 mg) as a yellow solid. LCMS (ESI) m/z=353 $(M+H)^+$. ^1H NMR (500 MHz, MeOD) δ (ppm) 8.79-8.89 (m, 4H), 7.98-8.20 (m, 4H), 5.04 (s, 2H), 4.85 (s, 2H), 3.90-3.95 (m, 3H), 3.50 (s, 1H), 3.33 (s, 2H).

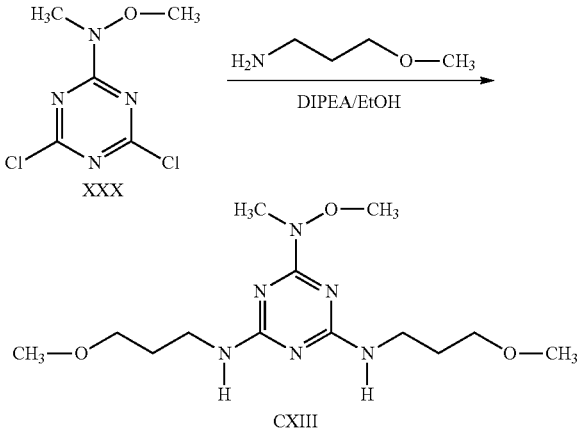
Example 57

4,6-Bis-[N-(3-methoxy-n-propyl)amino]-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CXIII)

Example 58

4,6-Bis-[N-(3-methoxy-n-propyl)amino]-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride salt (CXIV)

Scheme 42



4,6-Bis-[N-(3-methoxy-n-propyl)amino]-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CXIII)

N-(4,6-Dichloro[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXX) (1 g, 4.78 mmol), 3-methoxypropan-1-amine (936 mg, 10.52 mmol) and DIPEA (1.85 g, 14.34 mmol) in EtOH (50 mL) were heated at 100° C. for 16 h, after which time the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (200 mL), washed with water (50 mL) and then with a brine solution (50 mL) and lastly dried over Na_2SO_4. The solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (pet ether/ethyl acetate=5/1 to 1/2) to yield 4,6-bis-[N-(3-methoxy-n-propyl)amino]-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CXIII) (1.4 g, 93%).

4,6-Bis-[N-(3-methoxy-n-propyl)amino]-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (CXIV)

4,6-Bis-[N-(3-methoxy-n-propyl)amino]-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CXIII, 1.4 g, 4.46 mmol) was dissolved in H_2O (10 mL) and 0.5 M aqueous HCl solution (13.4 mL), and the resultant solution was lyophilized to yield 4,6-bis-[N-(3-methoxy-n-propyl)amino]-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (1.56 g) as a colorless oil. LCMS (ESI) m/z=315 $(M+H)^+$. ^1H NMR (500 MHz, DMSO) δ (ppm) 12.10-12.60 (br, 1H),

8.55-8.74 (m, 2H), 3.75-3.86 (m, 3H), 3.35-3.45 (m, 11H), 3.22-3.25 (m, 5H), 2.77 (s, 1H), 1.72-1.76 (m, 4H).

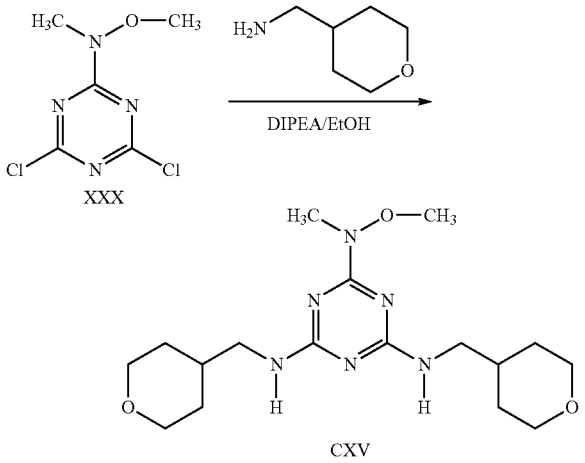
Example 59

4,6-Bis-[N-(tetrahydropyran-4-ylmethyl)amino]-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CXV)

Example 60

4,6-Bis-[N-(tetrahydropyran-4-ylmethyl)amino]-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (CXVI)

Scheme 43



4,6-Bis-[N-(tetrahydropyran-4-ylmethyl)amino]-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CXV)

N-(4,6-Dichloro[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXX) (1 g, 4.78 mmol), (tetrahydro-2H-pyran-4-yl)methanamine (1.21 g, 10.52 mmol) and DIPEA (1.85 g, 14.34 mmol) in EtOH (50 mL) were heated at 100° C. for 16 h, after which time the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (200 mL), washed with water (50 mL) and then with a brine solution (50 mL) and lastly dried over Na_2SO_4. The solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (PE/EA=5/1 to 1/1) to afford 4,6-bis-[N-(tetrahydropyran-4-ylmethyl)amino]-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CXV) (1.5 g, 85%).

4,6-Bis-[N-(tetrahydropyran-4-ylmethyl)amino]-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine hydrochloride (CXVI)

4,6-Bis-[N-(tetrahydropyran-4-ylmethyl)amino]-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CXV) (1.5 g, 4.1 mmol) was dissolved in H_2O (10 mL) and 0.5 M aqueous HCl solution (12.3 mL), and the resultant solution was lyophilized to yield 4,6-bis-[N-(tetrahydropyran-4-ylmethyl)amino]-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (CXVI) hydrochloride (1.65 g) as a white solid. LCM: (ESI) m/z=367 $(M+H)^+$. ^1H NMR (500 MHz, DMSO) δ (ppm) 12.30-12.70 (br, 1H), 8.65-8.80 (m, 2H), 3.77-3.86 (m, 7H), 3.20-3.35 (m, 11H), 1.75-1.78 (m, 2H), 1.56-1.58 (m, 4H), 1.20-1.23 (m, 4H).

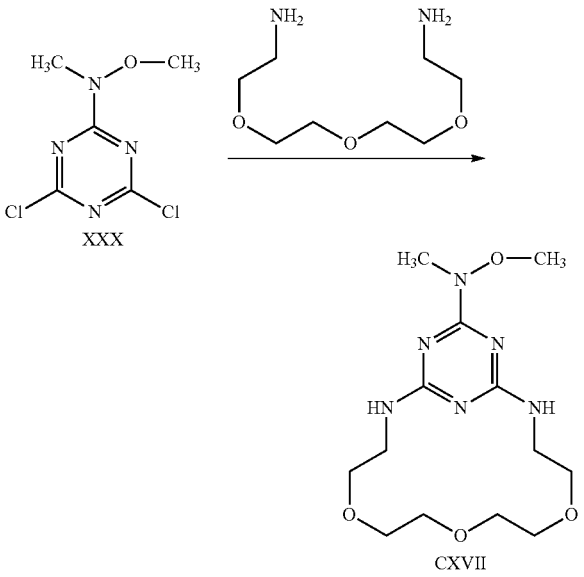
Example 61

N-(5,8,11-Trioxa-2,14,16,18,19-pentaazabicyclo[13.3.1]nonadeca-1(18),15 (19),16(17)-trien-17-yl)-N,O-dimethylhydroxylamine (CXVII)

Example 62

N-(5,8,11-Trioxa-2,14,16,18,19-pentaazabicyclo[13.3.1]nonadeca-1(18),15 (19),16(17)-trien-17-yl)-N,O-dimethylhydroxylamine hydrochloride (CX-VIII)

Scheme 44



N-(5,8,11-Trioxa-2,14,16,18,19-pentaazabicyclo[13.3.1]nonadeca-1(18),15(19),16(17)-trien-17-yl)-N,O-dimethylhydroxylamine (CXVII)

N-(4,6-Dichloro[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXX) (1.63 g, 7.8 mmol) in EtOH (100 mL) was added to 2,2'-(2,2'-oxybis(ethane-2,1-diyl)bis(oxy))di-ethanamine (*Org. Biomol. Chem.* 2005, 3:2255-61) (1.5 g, 7.8 mmol) and DIPEA (2.01 g, 15.6 mmol). The reaction was heated at 100° C. for 3 h, after which time the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (200 mL), washed with water (2×100 mL) and then with a brine solution (100 mL) and lastly dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (DCM/MeOH=50/1 to 20/1) to yield N-(5,8,11-trioxa-2,14,16,18,19-pentaazabicyclo[13.3.1]nonadeca-1(18),15(19),16(17)-trien-17-yl)-N,O-dimethylhydroxylamine (CXVII) (700 mg) as a colourless oil (yield 27%).

N-(5,8,11-Trioxa-2,14,16,18,19-pentaazabicyclo [13.3.1]nonadeca-1(18),15(19),16(17)-trien-17-yl)-N,O-dimethylhydroxylamine hydrochloride (CX-VIII)

N-(5,8,11-Trioxa-2,14,16,18,19-pentaazabicyclo[13.3.1] nonadeca-1(18),15(19),16(17)-trien-17-yl)-N,O-dimethyl-hydroxylamine (CXVII) (700 mg, 2.1 mmol) was dissolved in H_2O (10 mL) and 0.5 M aqueous HCl solution (4.3 mL), and the resultant solution was lyophilized to yield N-(5,8,11-trioxa-2,14,16,18,19-pentaazabicyclo[13.3.1]nonadeca-1 (18),15(19),16(17)-trien-17-yl)-N,O-dimethylhydroxy-lamine hydrochloride (CXVIII) (750) mg as a colorless oil. LCMS: (ESI) m/z=329 $(M+H)^+$. ^1H NMR (500 MHz, DMSO) δ (ppm) 11.50-12.60 (br, 1H), 8.61 (s, 2H), 3.77 (s, 3H), 3.30-3.62 (m, 16H), 3.30 (s, 3H).

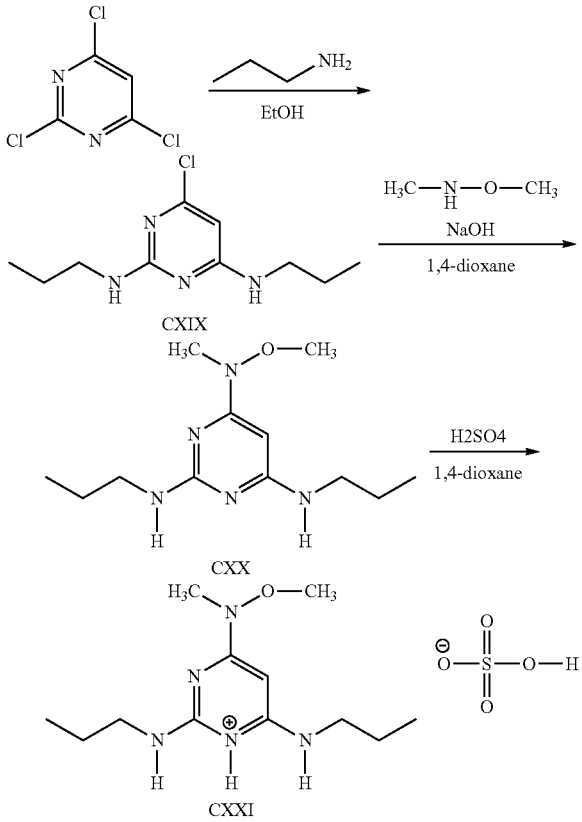
Example 63

2,6-Bis-(N-n-propylamino)-[1,3]pyrimidin-4-yl)-N, O-dimethyl-hydroxylamine (CXX)

Example 64

2,6-Bis-(N-n-propylamino)-[1,3]pyrimidin-4-yl)-N, O-dimethyl-hydroxylamine hydrogen sulfate (CXXI)

Scheme 45



4-Chloro-2,6-bis-[N-n-propylamino]-1,3-pyrimidine (CXIX)

2,4,6-Trichloro-pyrimidine (5.00 g, 27.26 mmol) and n-propylamine (13.5 mL, 163.56 mmol) in EtOH were heated at 60° C. for 24 h and then cooled. The volatiles were removed under reduced pressure. Water (100 mL) was added and the resulting suspension was extracted with CH_2Cl_2 (3×75 mL). The combined organic extracts were washed with water (150 mL), then with a brine solution (100 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure to yield 4-chloro-2,6-bis-[N-n-propylamino]-1,3-pyrimidine (CXIX) (5.78 g, 93%). 200 MHz ^1H-NMR (DMSO-d_6, ppm) 7.26-7.04 (1H, m) 7.04-6.81 (1H, m) 5.69 (1H, s) 3.26-3.01 (4H, m) 1.60-1.36 (4H, m) 0.87 (3H, t, J=7.4 Hz) 0.85 (3H, t, J=7.4 Hz); ESI-MS (m/z) 229, 231 $[M+H]^+$.

(2,6-Bis-N-[n-propylamino]-pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine (CXX)

4-Chloro-2,6-bis-[N-n-propylamino]-1,3-pyrimidine (CXIX) (5.78 g, 25.27 mmol), N,O-dimethylhydroxylamine hydrochloride (4.93 g, 50.54 mmol) and NaOH (2.02 g, 50.54 mmol) in 1,4-dioxane (400 mL) and water (20 mL) were heated at 60° C. for 24 h. N,O-Dimethylhydroxylamine hydrochloride (4.93 g, 50.54 mmol) and NaOH (3.03 g, 75.81 mmol) were added to the reaction mixture and heating was continued for 3 days at 110° C. The volatiles were removed under reduced pressure. Saturated $NaHCO_3$ solution (50 mL) was added to the residue and the mixture was extracted with CH_2Cl_2 (3×75 mL). The combined organic extracts were washed with water (150 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography (CH_2Cl_2/EtOH (25/1)) to yield (2,6-bis-N-[n-propylamino]-pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine (CXX) (1.2 g, 19%). 200 MHz ^1H-NMR (DMSO-d_6, ppm) 6.57-6.45 (1H, m) 6.13 (1H, t, J=5.5 Hz) 5.34 (1H, s) 3.59 (3H, s) 3.20-3.04 (4H, m) 3.03 (3H, s) 1.58-1.37 (4H, m) 0.87 (3H, t, J=7.1 Hz) 0.84 (3H, t, J=7.1 Hz).

(2,6-Bis-N-[n-propylamino]-pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine hydrogen sulfate (CXXI)

(2,6-Bis-N-[n-propylamino]-pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine (1.20 g, 4.74 mmol) in 1,4-dioxane (15 mL) was treated with 95% H_2SO_4 (0.27 mL, 4.74 mmol) in a dropwise manner at 0° C. The mixture was stirred at room temperature for 0.5 h and then the volatiles were removed under reduced pressure. The resulting residue was co-evaporated with dry toluene (3×5 mL) to yield (2,6-bis-N-[n-propylamino]-pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine hydrogen sulfate (CXXI) in quantitative yield. 400 MHz H-NMR (DMSO-d_6, ppm) 11.3-10.5 (1H, m), 8.29 (0.4H, br s), 7.38 (0.6H, br s), 5.48-5.20 (1H, m), 3.70 (3H, s), 3.36-3.21 (5H, m), 3.20-3.08 (2H, m), 1.61-1.48 (4H, m), 0.9 (6H, t, J=7.4 Hz). ESI-MS (m/z) 254 $[M+H]^+$; melting point: 123-126° C.

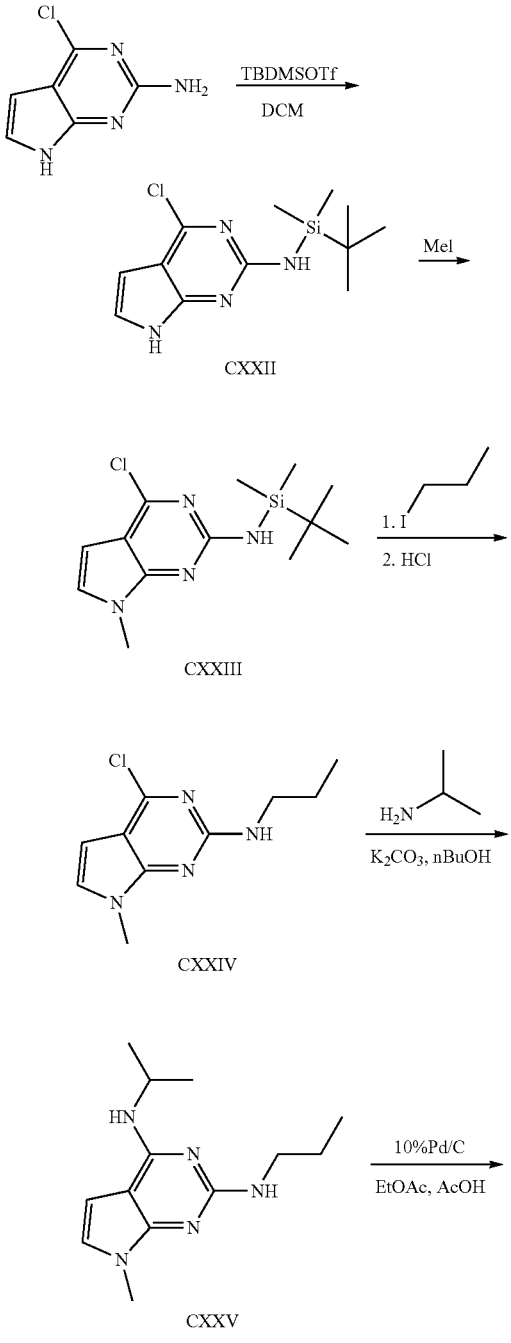
Example 65

2-(n-Propyl)amino-4-(i-propylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine (CXXVI)

Example 66

2-(n-Propyl)amino-4-(i-propylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine hydrochloride (CXXVII)

Scheme 46



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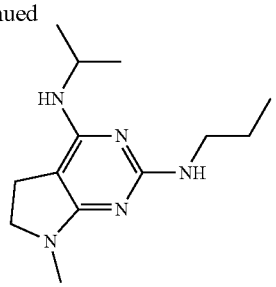


CXXVI

2-(t-Butyl-dimethylsilyl)amino-4-chloro-7H-pyrrolo[2,3-d]pyrimidine (CXXII)

To a dichloromethane solution (100 mL) of 2-amino-4-chloro-7H-pyrrolo[2,3-d]pyrimidine (8.7 g, 52 mmol) was added Et_3N (26 g, 260 mmol) and the mixture was stirred at −30° C. After this time, TBDMSOTf (15.1 g, 57.2 mmol) was added in a slow dropwise manner and the resultant reaction was stirred at ambient temperature for 1.5 h. The observed solid material completely dissolved to form a light brown solution. The mixture was then quenched with 1 N NaOH (100 mL) and extracted with DCM (250 mL). The organic layer was washed with H_2O (150 mL) and then with a brine solution (150 mL) and dried over Na_2SO_4. The solvents were removed in vacuo and the residue was purified by flash column chromatography (PE/EtOAc=10/1 to 5/1) to afford 2-(t-butyl-dimethylsilyl)amino-4-chloro-7H-pyrrolo[2,3-d]pyrimidine (CXXII, 11.4 g, 79%) as a light yellow solid. LCMS: (ESI) m/z=283 $(M+H)^+$.

2-(t-Butyl-dimethylsilyl)amino-4-chloro-7-methyl-pyrrolo[2,3-d]pyrimidine (CXXIII)

2-(t-Butyl-dimethylsilyl)amino-4-chloro-7H-pyrrolo[2,3-d]pyrimidine (CXXII) (700 mg, 2.5 mmol), 0.18 mL (3.5 mmol) of methyl iodide in DMF (10 mL), and K_2CO_3 (552 mg, 4 mmol) were reacted at ambient temperature for 15 h. After addition of H_2O (10 mL), the reaction was extracted with EtOAc (100 mL), and the organic layer was washed with a brine solution (10 mL), and dried over Na_2SO_4. The solvents were removed in vacuo, and the residue was purified by flash column chromatography (PE/EtOAc=10/1 to 5/1) to afford 2-(t-butyl-dimethylsilyl)amino-4-chloro-7-methyl-pyrrolo[2,3-d]pyrimidine (CXXIII) (750 mg, 100%) as a light yellow oil. LCMS (ESI) m/z=297 $(M+H)^+$.

2-(n-Propyl)amino-4-chloro-7-methyl-pyrrolo[2,3-c]pyrimidine (CXXIV)

Under a nitrogen atmosphere 2-(t-butyl-dimethylsilyl)amino-4-chloro-7-methyl-pyrrolo[2,3-d]pyrimidine (CXXIII, 5.0 g, 17 mmol) and 1-iodopropane (4.3 g, 25 mmol) were dissolved in DMF (20 mL). The reaction mixture was cooled to 0° C. with vigorous stirring, then NaH (1 g of a 60% dispersion in mineral oil, 21 mmol) was added. The mixture was stirred for 10 min. and water (50 mL) was slowly added to quench the reaction. The aqueous solution was extracted with EtOAc (200 mL), and the organic layer was washed with water (50 mL) and with a brine solution (50 mL), and lastly, dried over anhydrous Na_2SO_4. After evaporation of

volatiles, a yellow oily residue (5.7 g) was isolated. The residue was dissolved in Et_2O (50 mL) and concentrated hydrochloric acid (10 mL) was added at 0° C. with stirring. The mixture was reacted for an additional 10 min. After this time, the solution was extracted with EtOAc (200 mL) and 1N NaOH (200 mL). The organic layer was washed with H_2O (150 mL) and then with a brine solution (150 mL) and lastly, dried over Na_2SO_4. The solvents were removed in vacuo and the resultant residue was purified by flash column chromatography (PE/EtOAc=10/1 to 5/1) to afford 2-(n-propyl) amino-4-chloro-7-methyl-pyrrolo[2,3-d]pyrimidine (3.78 g, 100%) of the desired product as a yellow solid. LCMS: (ESI) m/z=225 $(M+H)^+$.

2-(n-Propyl)amino-4-(i-propyl)amino-7-methyl-pyrrolo[2,3-d]pyrimidine (CXXV)

To a solution of 2-(n-propyl)amino-4-chloro-7-methyl-pyrrolo[2,3-d]pyrimidine (1.6 g, 7.1 mmol) in n-butanol (10 mL) was added potassium carbonate (4.9 g, 35.5 mmol), followed by propan-2-amine (632 mg, 10.7 mmol). The mixture was stirred in an autoclave equipped with a stirrer at 140° C. for 16 h. After cooling to room temperature, water was added (20 mL), and the mixture was extracted with EtOAc (3×50 mL). The combined organic layers were washed with water and then with a brine solution, and lastly dried over anhydrous Na_2SO_4. The volatiles were removed in vacuo and the residue was purified by flash column chromatography (PE/EtOAc=3/1) to yield 2-(n-propyl)amino-4-(i-propyl) amino-7-methyl-pyrrolo[2,3-d]pyrimidine (1.2 g, 75%) as a yellow solid. LCMS: (ESI) m/z=248 $(M+H)^+$.

2-(n-Propyl)amino-4-(i-propylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine (CXXVI)

To the solution of 2-(n-propyl)amino-4-(i-propyl)amino-7-methyl-pyrrolo[2,3-d]pyrimidine (CXXV, 1.0 g, 4 mmol) in EtOAc (50 mL) were added 10% Pd/C (1.0 g) and AcOH (2.43 g, 40 mmol). The mixture was attached to a hydrogenation apparatus and the system was evacuated and then refilled with hydrogen. The reaction was stirred at ambient temperature for 48 h. After this time, the mixture was filtered over 10 g of silica-gel on a glass-filter. The filtrate was concentrated and the residue was purified by flash column chromatography (DCM/MeOH=50/1 to 10/1) to yield 2-(n-propyl)amino-4-(i-propylamino-7-methyl-pyrrolidino[2,3-d] pyrimidine (CXXVI, 500 mg, 50%) as a yellow solid.

2-(n-Propyl)amino-4-(i-propylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine hydrochloride (CXXVII)

The isolated free amine (CXXVI, 500 mg, 2 mmol) was dissolved in H_2O (10 mL) and 0.5 M aqueous HCl solution (4 mL), and the solution was lyophilized to yield 2-(n-propyl) amino-4-(i-propylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine hydrochloride (CXXVII, 525 mg) as a brown solid. LCMS (ESI) m/z=250 $(M+H)^+$. ^1H NMR (500 MHz, MeOD) δ (ppm) 3.80 (s, 1H), 3.58 (t, J=8.5 Hz, 2H), 3.23-3.29 (m, 2H), 2.88 (s, 3H), 2.77 (t, J=8.5 Hz, 2H), 1.52-1.56 (m, 2H), 1.15 (d, J=6.5 Hz, 6H), 0.89 (d, J=7.5 Hz, 3H).

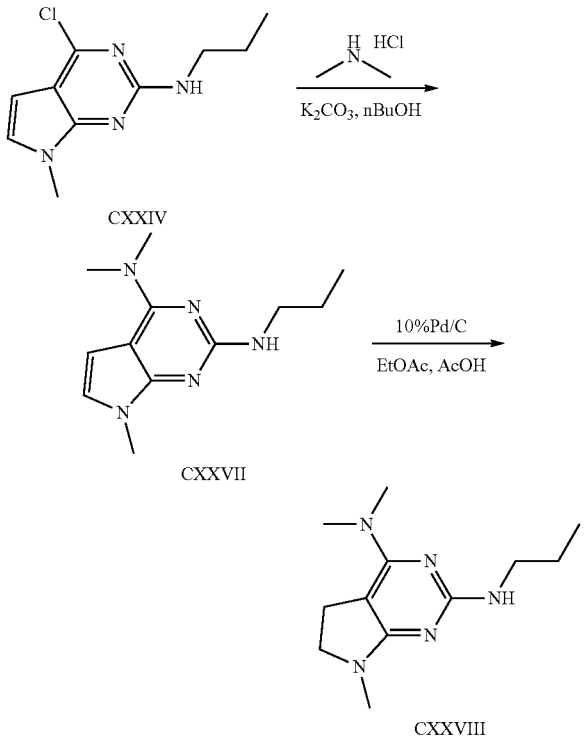
Example 67

2-(n-Propyl)amino-4-dimethylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine (CXXVIII)

Example 68

2-(n-Propyl)amino-4-dimethylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine hydrochloride (CXXIX)

Scheme 47



2-(n-Propyl)amino-4-dimethylamino-7-methyl-pyrrolo[2,3-d]pyrimidine (CXXVII)

To 2-(n-propyl)amino-4-chloro-7-methyl-pyrrolo[2,3-d] pyrimidine (1.0 g, 4.5 mmol) (CXXIV) in n-butanol (20 mL) was added potassium carbonate (3.7 g, 27 mmol) and dimethylamine hydrochloride (1.0 g (22 mmol). The mixture was stirred in an autoclave equipped with a stirrer at 120° C. for 16 h. After cooling to room temperature, water was added (20 mL), and the mixture was extracted with EtOAc (3×50 mL). The combined organic layers were washed with water and then with a brine solution, and dried over anhydrous Na_2SO_4. The solvents were removed in vacuo and the residue was purified by flash column chromatography (PE/EtOAc=6/1) to yield 2-(n-propyl)amino-4-dimethylamino-7-methyl-pyrrolo [2,3-d]pyrimidine (CXXVII, 800 mg, 76%) as a yellow solid. LCMS (ESI) m/z=234 $(M+H)^+$.

2-(n-Propyl)amino-4-dimethylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine (CXXVIII)

To the solution of 2-(n-propyl)amino-4-dimethylamino-7-methyl-pyrrolo[2,3-d]pyrimidine (CXXVII, 800 mg, 3.4

mmol) in EtOAc (30 mL) was added 10% Pd/C (1.0 g) and AcOH (3 mL), and the mixture was attached to a hydrogenation apparatus. The system was evacuated and then refilled with hydrogen. The mixture was stirred at ambient temperature for 48 h. The mixture was filtered through 10 g of silica-gel on a glass-filter. The filtrate was concentrated and the residue was purified by flash column chromatography (EtOAc/MeOH=25/1) to yield 2-(n-propyl)amino-4-dimethylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine (CXXVIII, 600 mg, 75%).

2-(n-Propyl)amino-4-dimethylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine hydrochloride (CXXIX)

2-(n-Propyl)amino-4-dimethylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine (CXXVIII, 600 mg, 2.6 mmol) was dissolved in H_2O (10 mL) and 0.5 M aqueous HCl solution (5.2 mL), and the solution was lyophilized to yield 2-(n-propyl)amino-4-dimethylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine hydrochloride (CXXIX, 600 mg) as a yellow solid. LCMS (ESI) m/z=236 $(M+H)^+$. ^1H NMR (500 MHz, MeOD) δ (ppm) 3.66 (t, J=9.0 Hz, 2H), 3.40 (t, J=7.0 Hz, 2H), 3.33 (s, 1H), 3.23 (t, J=9.5 Hz, 2H), 3.17 (s, 5H), 3.00 (s, 3H), 1.63-1.67 (m, 2H), 1.00 (t, J=7 Hz, 3H).

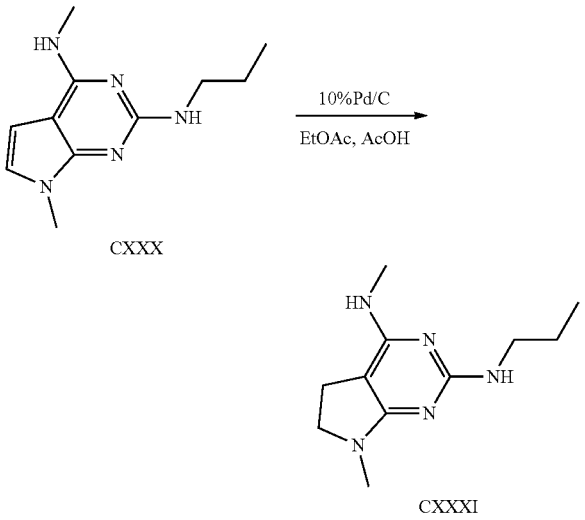
Example 69

2-(n-Propyl)amino-4-methylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine (CXXXI)

Example 70

2-(n-Propyl)amino-4-methylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine hydrochloride (CXXXII)

Scheme 48



2-(n-Propyl)amino-4-methylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine (CXXXI)

To a solution of 2-(n-propyl)amino-4-methylamino-7-methyl-pyrrolo[2,3-d]pyrimidine (CXXX) (2.0 g, 9.13 mmol) in EtOAc (50 mL) was added 10% Pd/C (2.1 g) and AcOH (8 mL), and the mixture was attached to a hydrogenation apparatus. The system was evacuated and refilled with hydrogen. The mixture was stirred at ambient temperature for 48 h. The mixture was filtered through 10 g of silica-gel on a glass-filter. The filtrate was concentrated and the residue was purified by flash column chromatography (DCM/MeOH=60/1 to 10/1) to yield 2-(n-propyl)amino-4-methylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine (CXXXI, 700 mg, 43%) as a yellow solid.

2-(n-Propyl)amino-4-methylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine hydrochloride (CXXXII)

2-(n-Propyl)amino-4-methylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine (CXXX, 700 mg, 3.2 mmol) was dissolved in H_2O (10 mL) and 0.5 M aqueous HCl solution (7 mL), and the solution was lyophilized to yield 2-(n-propyl)amino-4-methylamino-7-methyl-pyrrolidino[2,3-d]pyrimidine (717 mg) as a brown solid. LCMS: (ESI) m/z=222 $(M+H)^+$. ^1H NMR (500 MHz, MeOD) δ (ppm) 3.50 (t, J=8.5, 2H), 3.22 (t, J=7.0 Hz, 2H), 2.79 (s, 6H), 2.68 (t, J=9.0 Hz, 2H), 1.43-1.51 (m, 2H), 0.81 (t, J=8.0 Hz, 3H).

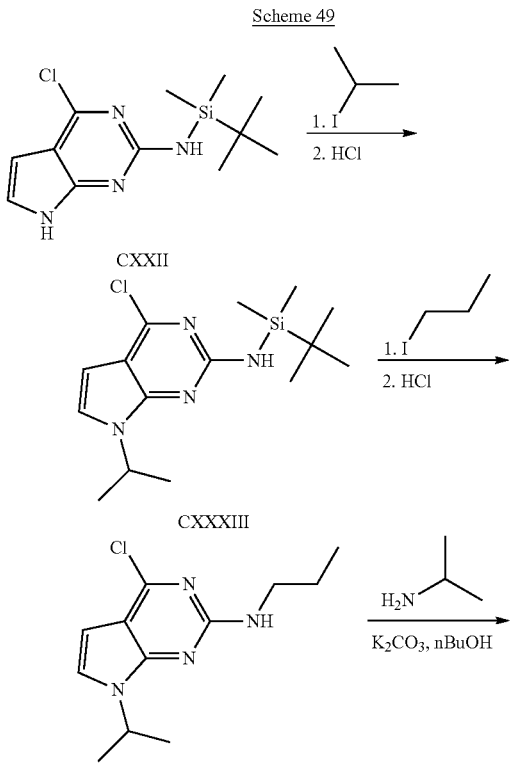
Example 71

2-(n-Propyl)amino-4-(i-propyl)amino-7-i-propyl-pyrrolidino[2,3-d]pyrimidine (CXXXVI)

Example 72

2-(n-Propyl)amino-4-(i-propyl)amino-7-i-propyl-pyrrolidino[2,3-d]pyrimidine hydrochloride salt (CXXXVII)

Scheme 49



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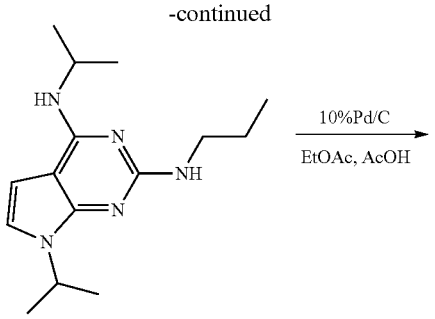
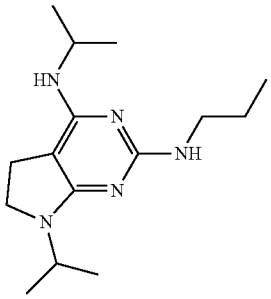


CXXXV



CXXXVI

2-(t-Butyl-dimethylsilyl)amino-4-chloro-7-i-propyl-pyrrolo[2,3-d]pyrimidine (CXXXIII)

2-(t-Butyl-dimethylsilanyl)amino-4-chloro-7H-pyrrolo[2,3-d]pyrimidine (CXXII) (5 g, 17.7 mmol), 2-iodopropane (4.5 g, 26.6 mmol) and K_2CO_3 (4.3 g, 26.6 mmol) in DMF (20 mL) were reacted at ambient temperature for 15 h. After addition of H_2O (50 mL) to the reaction mixture, the aqueous solution was extracted with EtOAc (300 mL) and the organic layer was washed with a brine solution (50 mL) and dried with Na_2SO_4. The solvents were removed in vacuo and the residue was purified by flash column chromatography (pet ether/EtOAc=10/1 to 5/1) to afford 2-(t-butyl-dimethylsilyl) amino-4-chloro-7-i-propyl-pyrrolo[2,3-d]pyrimidine (CXXXIII, 5.7 g, 100%) as a light yellow oil. LCMS: (ESI) m/z=325 $(M+H)^+$.

2-(n-Propyl)amino-4-chloro-7-i-propyl-pyrrolo[2,3-c]pyrimidine (CXXXIV)

Under nitrogen atmosphere, 2-(t-butyl-dimethylsilanyl) amino-4-chloro-7-1-propyl-pyrrolo[2,3-d]pyrimidine (5.7 g, 17.6 mmol) and 1-iodopropane (4.5 g (26.4 mmol) were dissolved in DMF (20 mL). The reaction mixture was cooled to 0° C. with vigorous stirring, and NaH (1.06 g of a 60% dispersion in mineral oil, 26.4 mmol) of (60%) was added. The mixture was stirred for 10 min, and then water (50 mL) was slowly added to quench the reaction. The mixture was extracted with EtOAc (300 mL) and the organic layer was washed with water (50 mL) and then with a brine solution (50 mL), and lastly, dried over anhydrous Na_2SO_4. After evaporation of the solvents, a yellow oily residue was isolated (6.5 g). The residue was dissolved in Et_2O (50 mL), concentrated hydrochloric acid (10 mL) was added at 0° C. with stirring, and the mixture was stirred for an additional 10 min. After the reaction was completed, the solution was extracted with EtOAc (200 mL) and 1N NaOH (200 mL). The organic layer was washed with H_2O (150 mL) and then with a brine solution (150 mL) and dried over Na_2SO_4. The solvents in vacuo and the residue was purified by flash column chromatography (PE/EtOAc=10/1 to 5/1) to yield 2-(n-propyl)amino-4-chloro-7-i-propyl-pyrrolo[2,3-d]pyrimidine (CXXXIV, 4.5 g, !00%) as a yellow solid. LCMS (ESI) m/z=253 $(M+H)^+$.

2-(n-Propyl)amino-4-(i-propyl)amino-7-i-propyl-pyrrolo[2,3-c]pyrimidine (CXXXV)

To 2-(n-propyl)amino-4-chloro-7-i-propyl-pyrrolo[2,3-d] pyrimidine (CXXXIV, 3.0 g, 12 mmol) in n-butanol (10 mL) was added propan-2-amine (1.05 g, 18 mmol) and potassium carbonate (2.5 g, 18 mmol). The resulting mixture was stirred in an autoclave equipped with a stirrer at 140° C. for 16 h. After cooling to room temperature, water was added (20 mL), and the mixture was extracted with EtOAc (3×50 mL). The combined organic layers were washed with water and then with a brine solution and dried over anhydrous Na_2SO_4. After the solvents were removed in vacuo, the residue was purified by flash column chromatography (PE/EtOAc=5/1 to 3/1) to yield 2-(n-propyl)amino-4-(i-propyl)amino-7-i-propyl-pyrrolo[2,3-d]pyrimidine (CXXXV, 1.2 g, 36%) as a yellow solid. LCMS: (ESI) m/z=276 $(M+H)^+$.

2-(n-Propyl)amino-4-(i-propyl)amino-7-i-propyl-pyrrolidino[2,3-d]pyrimidine (CXXXVI)

To a solution of 2-(n-propyl)amino-4-(i-propyl)amino-7-i-propyl-pyrrolo[2,3-d]pyrimidine (1.0 g, 3.6 mmol) in EtOAc (10 mL) were added 10% Pd/C (1.0 g) and AcOH (2.43 g, 40 mmol). The mixture was attached to a hydrogenation apparatus. The system was evacuated and refilled with hydrogen gas. The mixture was stirred at ambient temperature for 48 h, and filtered through 10 g of silica-gel on a glass-filter. The filtrate was concentrated and the residue was purified by flash column chromatography (DCM/MeOH=100/1 to 20/1) to yield 2-(n-propyl)amino-4-(i-propyl)amino-7-i-propyl-pyrrolidino[2,3-d]pyrimidine (CXXXVI) (800 mg, 79%) as a yellow solid.

2-(n-Propyl)amino-4-(i-propyl)amino-7-i-propyl-pyrrolidino[2,3-d]pyrimidine hydrochloride (CXXX-VII)

2-(n-Propyl)amino-4-(i-propyl)amino-7-i-propyl-pyrrolo [2,3-d]pyrimidine (200 mg, 0.72 mmol) was dissolved in H_2O (10 mL) and 0.5 M aqueous HCl solution (1.5 mL) and then the solution was lyophilized to yield 2-(n-propyl)amino-4-(i-propyl)amino-7-i-propyl-pyrrolidino[2,3-d]pyrimidine hydrochloride (225 mg, 95%) as a yellow solid. LCMS (ESI) m/z=278 $(M+H)^+$. ^1H NMR (500 MHz, MeOD) δ (ppm) 4.36 (s, 1H), 3.90 (s, 1H), 3.67 (t, J=8.5 Hz, 2H), 3.30 (t, J=7.5 Hz, 2H), 2.84 (t, J=8.5 Hz, 2H), 1.60-1.66 (m, 2H), 1.24 (d, J=6.0 Hz, 6H), 1.21 (d, J=6.0 Hz, 6H), 0.98 (t, J=7.5 Hz, 3H).

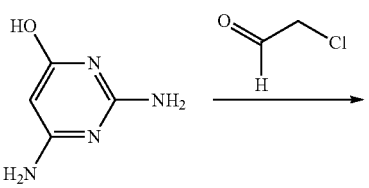
Example 73

N-(2-Propylamino-7H-pyrrolo[2,3d]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine (CXLI)

Example 74

N-(2-Propylamino-7H-pyrrolo[2,3d]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine hydrochloride (CX-LII)

Scheme 50



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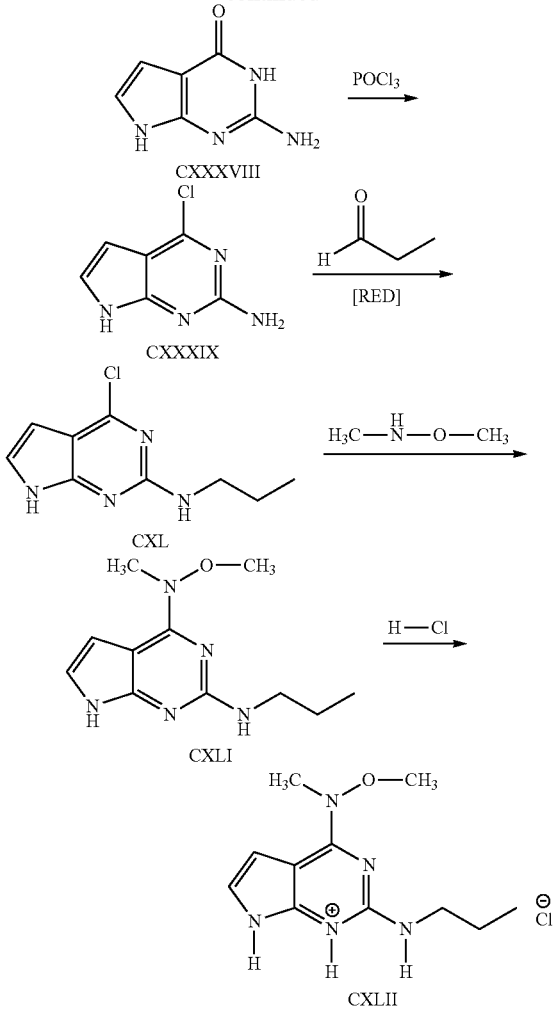


2-Amino-4-oxo-4,7-dihydro-3H-pyrrolo[2,3d]pyrimidine (CXXXVIII)

To the solution of 2,4-diamino-6-hydroxypyrimidine (50 g, 397 mmol) in H_2O (750 mL) was added 2-chloroacetaldehyde (40% in H_2O, 85 g, 437 mmol) in a dropwise manner at 0° C. The mixture was stirred at 65° C. for 2 h and then heated at 100° C. until the reaction was complete. The resultant solids were filtered and the remaining residue was heated at reflux in EtOH (750 mL). The additional solids were filtered and the mother liquor was concentrated to afford 2-amino-4-oxo-4,7-dihydro-3H-pyrrolo[2,3d]pyrimidine (CXXXVIII) as a yellow solid 40 g (~67%, ~70% purity). LCMS (ESI) m/z=151 $(M+H)^+$.

2-Amino-4-chloro-7H-pyrrolo[2,3d]pyrimidine (CXXXIX)

2-Amino-4-oxo-4,7-dihydro-3H-pyrrolo[2,3d]pyrimidine (CXXXVIII, 25 g, 167 mmol) was suspended in $POCl_3$ (200 mL) and cooled in an ice bath. The mixture was slowly warmed and heated up to 120° C. for 3 h. After this time, the volatiles (excess $POCl_3$) were evaporated the under vacuum. To this residue was added ice water (200 mL) and the resultant solid was filtered to afford 2-amino-4-chloro-7H-pyrrolo[2,3d]pyrimidine (CXXXIX) as a yellow solid (20 g, ~71%, ~75% purity). LCMS (ESI) m/z=169 $(M+H)^+$.

4-Chloro-2-n-propylamino-7H-pyrrolo[2,3d]pyrimidine (CXL)

To a solution of 2-amino-4-chloro-7H-pyrrolo[2,3d]pyrimidine (CXXXIX, 26 g, 155 mmol) and n-propionaldehyde (27 g, 464 mmol) in MeOH (600 mL) was added AcOH (50 mL). The reaction was stirred at ambient temperature for 30 min. After this time, $NaBH_3CN$ (49 g, 775 mmol) was added in portions at −20° C. for 30 min. The resultant mixture was stirred at 80° C. for 3 h and the volatiles were removed. The resultant residue was extracted with EtOAc (3×300 mL) and the combined organics were washed with a brine solution (2×100 mL). The organic layer was dried over Na_2SO_4, concentrated and purified via silica-gel column chromatography (pet ether/EtOAc (5/1)) to afford 4-chloro-2-n-propylamino-7H-pyrrolo[2,3d]pyrimidine (CXL) as a yellow solid (6 g, 18%). LCMS (ESI) m/z=211 $(M+H)^+$.

N-(2-Propylamino-7H-pyrrolo[2,3d]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine (CXLI)

To a solution of 4-chloro-2-n-propylamino-7H-pyrrolo[2,3d]pyrimidine (CXL, 1.0 g, 4.8 mmol) in n-BuOH (5 mL) was added potassium carbonate (3.3 g, 5.0 eq.) and N,O-dimethylhydroxylamine hydrochloride (1.1 g, 4.0 eq.). The mixture was stirred in an autoclave equipped with a stirrer at 100° C. for 8 h. After cooling to ambient temperature, water was added (20 mL) and the mixture was extracted with EtOAc (3×25 mL). The combined organic layers were washed with water and then with a brine solution, and dried over anhydrous Na_2SO_4. The solvents were removed in vacuo and the resultant residue was purified by flash column chromatography (pet ether/EtOAc=5/1) to yield N-(2-n-propylamino-7H-pyrrolo[2,3d]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine (CXLI, 500 mg, 45%).

N-(2-Propylamino-7H-pyrrolo[2,3d]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine hydrochloride (CXLII)

N-(2-n-propylamino-7H-pyrrolo[2,3d]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine (CXLI, 500 mg, 2.1 mmol) was dissolved in H_2O (10 mL) and 0.5 M aqueous HCl solution (5 mL). The solution was then lyophilized to yield N-(2-n-propylamino-7H-pyrrolo[2,3d]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine hydrochloride as a white solid (CXLII, 550 mg). LCMS (ESI) m/z=236 $(M+H)^+$. ^1H NMR (500 MHz, MeOD) δ (ppm) 6.74 (d, J=3.5 Hz, 1H), 6.41 (d, J=4.0 Hz, 1H), 3.80 (s, 3H), 3.35 (s, 3H), 3.28-3.35 (m, 2H), 1.59-1.62 (m, 2H), 0.97 (t, J=7.0 Hz, 3H).

Example 75

2,4-Bis-(n-propyl)amino-7H-pyrrolidino[2,3-d]pyrimidine (CXLIX)

Example 76

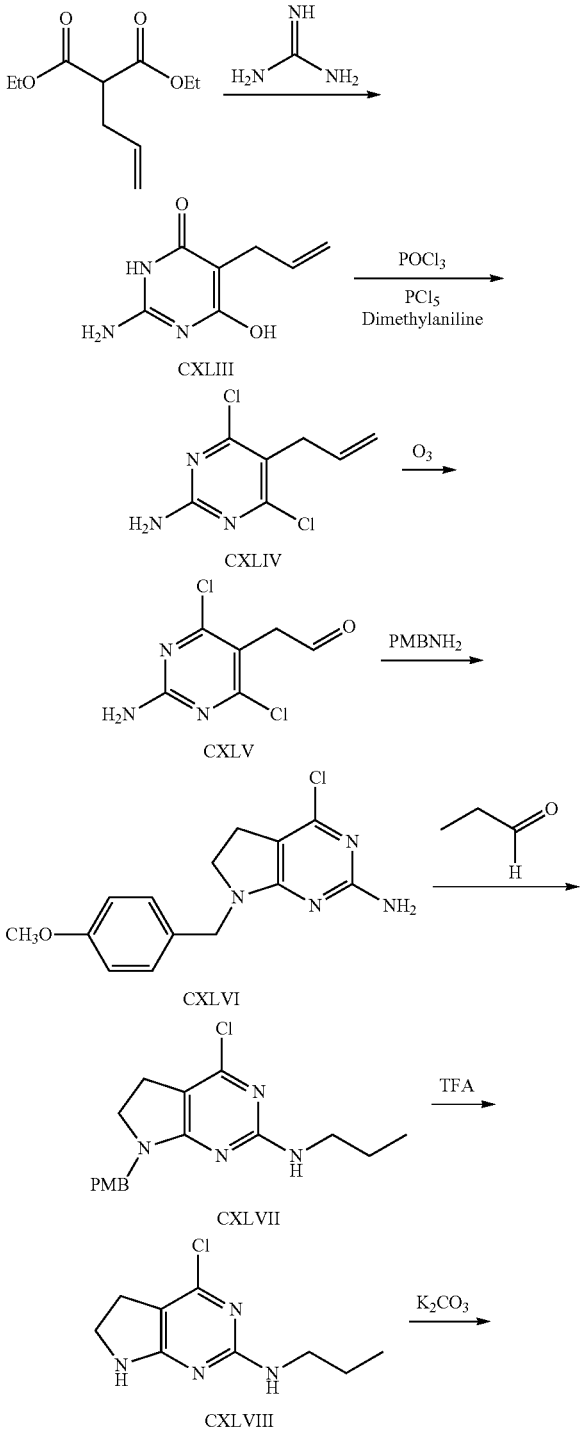
2,4-Bis-(n-propyl)amino-7H-pyrrolidino[2,3-d]pyrimidine hydrochloride (CL)

5-Allyl-2-amino-4,6-dihydroxypyrimidine (CXLIII)

Guanidine hydrochloride (27 g, 0.28 mol) was added to cold absolute EtOH (200 mL) and NaOEt (30% in ethanol)

(200 mL), and the mixture was stirred at 0° C. for 10 min. After this time, diethyl allylmalonate (55 g, 0.28 mol) was added. The reaction mixture was then stirred at room temperature for 18 h. Acidification with 3N HCl precipitated the crude product (pH=6). The solid was collected by filtration and washed with ethanol. Recrystallization from water afforded 29 g (62%) of pure 5-allyl-2-amino-4,6-dihydroxypyrimidine (CXLIII). LCMS (ESI) m/z=168 $(M+H)^+$.

Scheme 51



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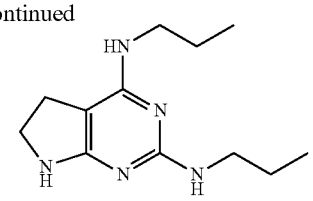


CXLIX

5-Allyl-2-amino-4,6-chloropyrimidine (CXLIV)

5-Allyl-2-amino-4,6-dihydroxypyrimidine (4.9 g, 28.0 mmol) was added in small portions to a solution of PCl_5 (6.6 g, 29.5 mmol) in $POCl_3$ (180 mL) at 60° C., and diethylaniline (3 g) was added dropwise. The temperature was raised to 120° C. The reaction mixture was heated at reflux overnight before it was evaporated to dryness. Hot water (100° C.) (100 mL) was added slowly to the residue, and the resulting suspension was cooled and extracted with CH_2Cl_2 (2×100 mL). The combined organic layers were washed with cold water three times until the aqueous extract was above pH=5. The organic layer was dried (Na_2SO_4) and evaporated to dryness in vacuo. The resultant residue was purified by column chromatography (EtOAc/pet ether=1:10) to afford 5-allyl-2-amino-4,6-chloropyrimidine (CXLIV, 2.5 g, 42%). LCMS (ESI) m/z=204 $(M+H)^+$.

2-(2-Amino-4,6-chloropyrimidin-5-yl)ethanal (CXLV)

5-Allyl-2-amino-4,6-chloropyrimidine (1 g, 4.9 mmol) was dissolved in ethyl acetate (40 mL) and reacted with ozone gas at −78° C. for about 1 h (about 5% ozone at a rate of 1 L/min). The reaction was monitored by TLC (pet ether/AcOEt=3/1 (v/v)), and once the starting material was consumed, the reaction mixture was flushed with oxygen for 10 min. At this time, NaI (3 g) and glacial acetic acid (3 mL) were added simultaneously to the cold reaction mixture, and the temperature was allowed to warm up to 20° C. with continuous stirring over a 60-min period. Sodium thiosulfate solution (67 g/100 mL of H_2O) was added to the reaction mixture until it became colorless. The resulting mixture was diluted with water (30 mL) and extracted with CH_2Cl_2 (4×70 mL). The combined organic extracts were washed successively with H_2O (4×30 mL), a saturated $NaHCO_3$ solution (30 mL) and with a brine solution (30 mL) and lastly, dried over anhydrous Na_2SO_4). After filtration and evaporation, 2-(2-amino-4,6-chloropyrimidin-5-yl)ethanal (CXLV, 1.1 g, 87%) was isolated as a white solid with ~80% purity. LCMS (ESI) m/z=207 $(M+H)^+$.

2-Amino-4-chloro-7-(4-methoxy)benzyl-pyrrolidino [2,3-d]pyrimidine (CXLVI)

A solution of 2-(2-amino-4,6-chloropyrimidin-5-yl)ethanal (CXLV, 1.2 g, 5.8 mmol) and para-methoxybenzylamine ($PMBNH_2$) (1.6 g, 11.6 mmol) in THF (20 mL) and AcOH (2 mL) was stirred at ambient temperature for 30 min. To this mixture was added $NaBH(OAc)_3$ (6.2 g, 29 mmol) in portions and the reaction was stirred overnight. The mixture was concentrated in vacuo, and extracted with EtOAc (3×80 mL). The combined organic extracts were then washed with a brine solution (2×50 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in vacuum. The resultant residue was puri-

fied by flash column chromatography (pet ether/EtOAc=10/1) to afford 2-amino-4-chloro-7-(4-methoxy)benzyl-pyrrolidino[2,3-d]pyrimidine (CXLVI, 950 mg, 52%) as a yellow solid (52%). LCMS (ESI) m/z=291 $(M+H)^+$.

2-n-Propylamino-4-chloro-7-(4-methoxy)benzyl-pyrrolidino[2,3-d]pyrimidine (CXLVII)

2-Amino-4-chloro-7-(4-methoxy)benzyl-pyrrolidino[2,3-d]pyrimidine (CXLVI, 950 mg, 3.3 mmol) and propionaldehyde (575 mg, 16.5 mmol) in MeOH (30 mL) and AcOH (3 mL) were stirred at ambient temperature for 30 min. At this time, $NaBH_3CN$ (1.0 g, 16.5 mmol) was added in portions and the reaction was then heated at 85° C. for 16 h. After cooling, the mixture was evaporated, and extracted with EtOAc (2×50 mL). The combined organics were then washed with a brine solution (2×50 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuum. The resultant residue was purified by flash column chromatography (PE/EtOAc=10/1) to afford 2-n-propylamino-4-chloro-7-(4-methoxy)benzyl-pyrrolidino[2,3-d]pyrimidine (CXLVII, 920 mg, 85%). LCMS (ESI) m/z=333 $(M+H)^+$.

2-n-Propylamino-4-chloro-7H-pyrrolidino[2,3-d]pyrimidine (CXLVIII)

A solution of 2-n-propylamino-4-chloro-7-(4-methoxy)benzyl-pyrrolidino[2,3-d]pyrimidine (CXLVII, 920 mg, 3.2 mmol) in TFA (5 mL) was heated at 85° C. for 3 h. After cooling the mixture was evaporated and extracted with EtOAc (2×50 mL). The combined organics were washed with a brine solution (2×50 mL), dried over Na_2SO_4, filtered and concentrated in vacuum. The resultant residue was purified by flash column chromatography (pet ether/EtOAc=5/1) to afford 2-n-propylamino-4-chloro-7H-pyrrolidino[2,3-d]pyrimidine (CXLVIII, 500 mg, 85%) as a colorless solid. LCMS (ESI) m/z=213 $(M+H)^+$.

2,4-Bis-(n-propyl)amino-7H-pyrrolidino[2,3-d]pyrimidine (CXLIX)

2-n-Propylamino-4-chloro-7H-pyrrolidino[2,3-d]pyrimidine (CXLVIII, 500 mg, 2.38 mmol) was dissolved in n-butanol (5 mL), and potassium carbonate (1.64 g, 5.0 eq.) and propan-1-amine (923 mg, 4.0 eq.) were added. The mixture was stirred in an autoclave equipped with a stirrer at 100° C. for 72 h. After cooling to room temperature, 20 ml, of water was added, and the mixture was extracted with EtOAc (3×20 mL). The combined organic layers were washed with water and with a brine solution, and dried over anhydrous Na_2SO_4. After removal of the solvents in vacuo, the resultant residue was purified by preparative HPLC to yield 2-n-propylamino-4-chloro-7H-pyrrolidino[2,3-d]pyrimidine (CXLIX, 210 mg, 37% yield).

2,4-Bis-(n-propyl)amino-7H-pyrrolidino[2,3-d]pyrimidine hydrochloride (CL)

2-n-Propylamino-4-chloro-7H-pyrrolidino[2,3-d]pyrimidine (CXLIX, 210 mg, 0.89 mmol) was dissolved in H_2O (5 mL) and 0.5 M aqueous HCl solution (2.0 mL), and the solution was lyophilized to yield 2,4-bis-(n-propyl)amino-7H-pyrrolidino[2,3-d]pyrimidine hydrochloride (CL, 242 mg, 95% yield) as an orange solid. LCMS (ESI) m/z=236 $(M+H)^+$. ^1H NMR (500 MHz, MeOD) δ (ppm) 3.72 (t, J=9.0 Hz, 2H), 3.30-3.34 (m, 4H), 2.90 (t, J=9.0 Hz, 2H), 1.61-1.65 (m, 4H), 0.96-0.99 (m, 6H).

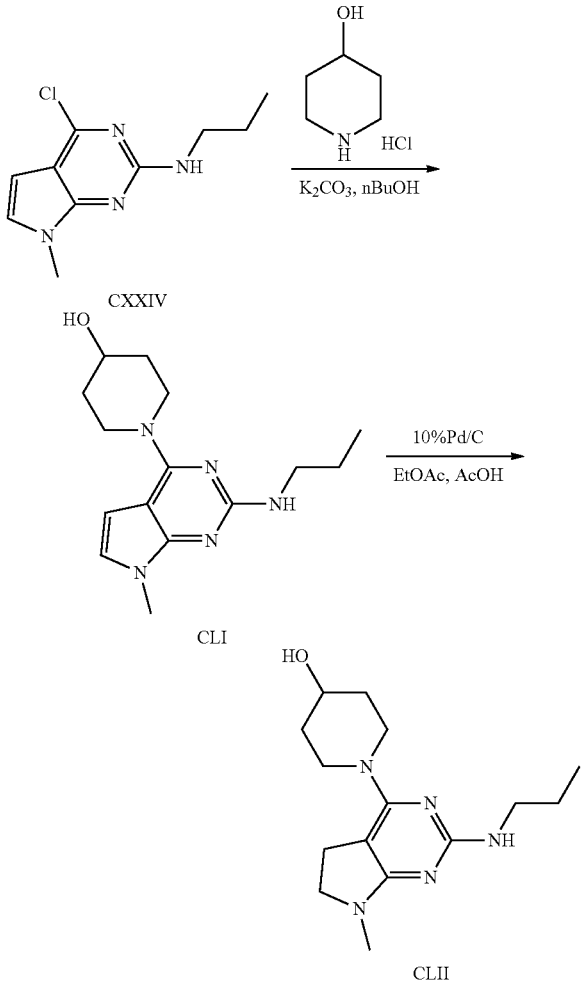
Example 77

2-(n-Propyl)amino-4-(4-hydroxypiperidin-1-yl)-7-methyl-pyrrolidino[2,3-d]pyrimidine (CLII)

Example 78

2-(n-Propyl)amino-4-(4-hydroxypiperidin-1-yl)-7-methyl-pyrrolidino[2,3-d]pyrimidine hydrochloride (CLIII)

Scheme 52



2-(n-Propyl)amino-4-(4-hydroxypiperidin-1-yl)-7-methyl-pyrrolo[2,3-d]pyrimidine (CLI)

2-(n-Propyl)amino-4-chloro-7-methyl-pyrrolo[2,3-d]pyrimidine (CXXIV) (1.6 g, 7.1 mmol) was added to n-butanol (10 mL) followed by potassium carbonate (4.9 g, 35.5 mmol) and piperidin-4-ol hydrochloride (632 mg, 10.7 mmol). The mixture was stirred in an autoclave equipped with a stirrer at 130° C. for 16 h. After cooling to room temperature, water was added (20 mL), and the mixture was extracted with EtOAc (3×50 mL). The combined organic layers were washed with water and then with a brine solution, and dried over anhydrous Na_2SO_4. The solvents were removed in vacuo

and the residue was purified by flash column chromatography (pet ether/EtOAc=3/1) to yield 2-(n-propyl)amino-4-(4-hydroxypiperidin-1-yl)-7-methyl-pyrrolo[2,3-d]pyrimidine (1.2 g, 75%) as a yellow solid. LCMS (ESI) m/z=290 $(M+H)^+$.

2-(n-Propyl)amino-4-(4-hydroxypiperidin-1-yl)-7-methyl-pyrrolidino[2,3-d]pyrimidine (CLII)

To the solution of 2-(n-propyl)amino-4-(4-hydroxypiperidin-1-yl)-7-methyl-pyrrolo[2,3-d]pyrimidine (CLI, 1.0 g, 4 mmol) in EtOAc (50 mL) were added 10% Pd/C (1.0 g) and AcOH (2.43 g, 40 mmol). The mixture was attached to a hydrogenation apparatus. The system was evacuated and refilled with hydrogen gas. The mixture was stirred at ambient temperature for 48 h. The mixture was filtered through 10 g of silica-gel on a glass-filter. The filtrate was concentrated and the residue was purified by flash column chromatography (DCM/MeOH=50/1 to 10/1) to yield 2-(n-propyl)amino-4-(4-hydroxypiperidin-1-yl)-7-methyl-pyrrolidino[2,3-d]pyrimidine (CLII, 500 mg, 50% yield) as a yellow solid.

2-(n-Propyl)amino-4-(4-hydroxypiperidin-1-yl)-7-methyl-pyrrolidino[2,3-d]pyrimidine hydrochloride (CLIII)

2-(n-Propyl)amino-4-(4-hydroxypiperidin-1-yl)-7-methyl-pyrrolidino[2,3-d]pyrimidine (CLII, 500 mg, 2 mmol) was dissolved in H_2O (10 mL) and 0.5 M HCl solution in H_2O (4 mL) and the solution was lyophilized to yield 2-(n-propyl) amino-4-(4-hydroxypiperidin-1-yl)-7-methyl-pyrrolidino[2,3-d]pyrimidine hydrochloride (525 mg) as a yellow solid. LCMS (ESI) m/z=292 $(M+H)^+$. ^1H NMR (500 MHz, MeOD) δ (ppm) 4.12-4.15 (m, 2H), 3.80-3.82 (m, 1H), 3.40 (t, J=8.0 Hz, 2H), 3.29 (t, J=7.0 Hz, 2H), 3.08-3.14 (m, 2H), 3.02 (t, J=8.0 Hz, 2H), 2.84 (s, 3H), 1.86-1.90 (m, 2H), 1.58-1.62 (m, 2H), 1.46-1.51 (m, 2H), 0.97 (t, J=4.0 Hz, 3H).

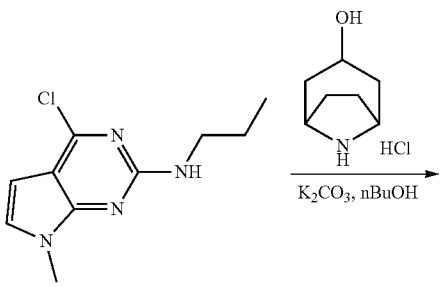
Example 79

8-(7-methyl-2-(n-propylamino)-pyrrolidino[2,3-d] pyrimidin-4-yl)-8-azabicyclo[3.2.1]octan-3-ol (CLV)

Example 80

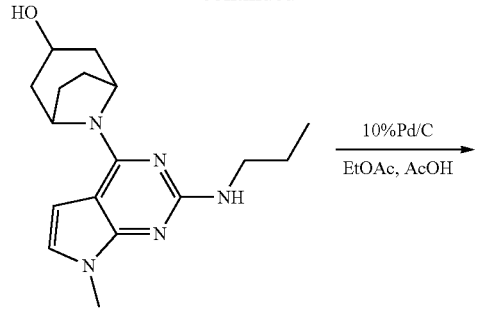
8-(7-methyl-2-(n-propylamino)-pyrrolidino[2,3-d] pyrimidin-4-yl)-8-azabicyclo[3.2.1]octan-3-ol hydrochloride (CLVI)

Scheme 53

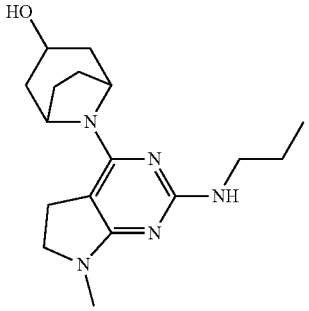


CXXIV

-continued



CLIV



CLV

8-(7-Methyl-2-(n-propylamino)-7H-pyrrolo[2,3-d] pyrimidin-4-yl)-8-azabicyclo[3.2.1]octan-3-ol (CLIV)

To a solution of 2-(n-propyl)amino-4-chloro-7-methyl-pyrrolo[2,3-d]pyrimidine (CXXIV) (1.0 g, 4.5 mmol) in n-butanol (10 mL) were added DIPEA (1.0 g, 7.8 mmol) and 8-aza-bicyclo[3.2.1]octan-3-ol hydrochloride (1.1 g, 6.7 mmol). The mixture was stirred at 125° C. for 16 h. After cooling to room temperature, water (20 mL) was added, and the mixture was extracted with EtOAc (3×50 mL). The combined organic layers were washed with water and then with a brine solution, and dried over anhydrous Na_2SO_4. The solvents were removed in vacuo and the resultant residue was purified by flash column chromatography (pet ether/EtOAc=6/1) to yield 8-(7-methyl-2-(n-propylamino)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-8-azabicyclo[3.2.1]octan-3-ol (CLIV) (800 mg, 57% yield) as a yellow solid. LCMS (ESI) m/z=316 $(M+H)^+$.

8-(7-Methyl-2-(n-propylamino)-pyrrolidino[2,3-d] pyrimidin-4-yl)-8-azabicyclo[3.2.1]octan-3-ol (CLV)

To a solution of 2-(n-propyl)amino-4-(4-hydroxy-1-azabicyclo[3.2.1]octan-1-yl)-7-methyl-pyrrolo[2,3-d]pyrimidine (CLIV, 1.00 g, 3.2 mmol) in EtOAc (10 mL) were added 10% Pd/C (1.0 g) and AcOH (3 mL). The mixture was attached to a hydrogenation apparatus. The system was evacuated and refilled with hydrogen. The mixture was stirred at ambient temperature for 48 h. The mixture was filtered through 10 g of silica-gel on a glass-filter. The filtrate was concentrated and the residue was purified by flash column chromatography (EtOAc/MeOH=50/1) to yield 8 (7-methyl-2-(n-propylamino)-pyrrolidino[2,3-d]pyrimidin-4-yl)-8-azabicyclo[3.2.1]octan-3-ol (CLV) (500 mg, 49% yield) as a yellow solid.

8-(7-Methyl-2-(n-propylamino)-pyrrolidino[2,3-d] pyrimidin-4-yl)-8-azabicyclo[3.2.1]octan-3-ol hydrochloride (CLVI)

8-(7-Methyl-2-(n-propylamino)-pyrrolidino[2,3-d]pyrimidin-4-yl)-8-azabicyclo[3.2.1]octan-3-ol (CLV, 200 mg, 0.63 mmol) was dissolved in H_2O (10 mL) and 0.5 M aqueous HCl solution (1.3 mL), and the solution was lyophilized to yield 8-(7-methyl-2-(n-propylamino)-pyrrolidino[2,3-d]pyrimidin-4-yl)-8-azabicyclo[3.2.1]octan-3-ol hydrochloride (CLVI) (224 mg) as a yellow solid. LCMS (ESI) m/z=318 $(M+H)^{+1}$. ^1H NMR (500 MHz, DMSO) δ (ppm) 4.55 (br, 2H), 3.90 (s, 1H), 3.60 (t, J=8.0 Hz, 2H), 3.27 (t, J=6.5 Hz, 2H), 3.00 (t, J=9.0 Hz, 2H), 2.92 (s, 3H), 2.27 (d, J=7.0 Hz, 2H), 1.90-1.95 (m, 4H), 1.69 (s, 1H), 1.66 (s, 1H), 1.49-1.54 (m, 2H), 0.92 (t, J=7.0 Hz, 3H).

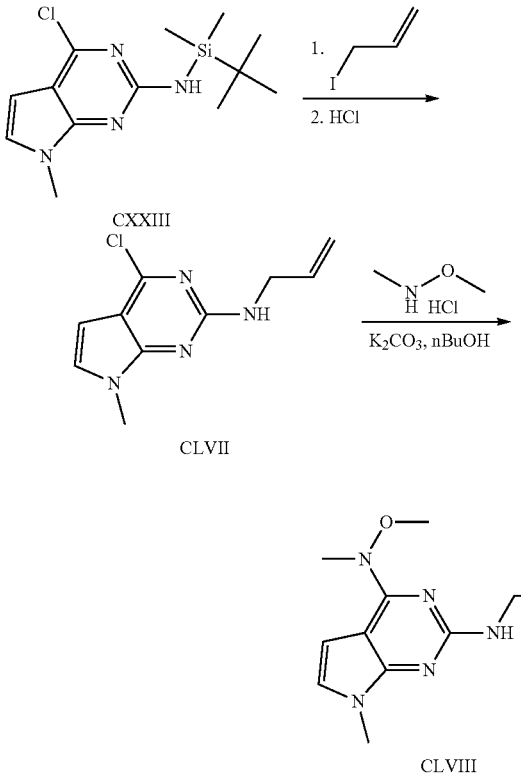
Example 81

N-(2-(Propen-2-yl)amino-7-methyl-pyrrolo[2,3d] pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine (CLVIII)

Example 82

N-(2-(Propen-2-yl)amino-7-methyl-pyrrolo[2,3d] pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine hydrochloride (CLIX)

Scheme 54



2-(Propen-2-yl)amino-4-chloro-7-methyl-pyrrolo[2, 3-d]pyrimidine (CLVII)

Under a nitrogen atmosphere, 2-(t-butyl-dimethylsilanyl) amino-4-chloro-7-methyl-pyrrolo[2,3-d]pyrimidine (CXXIII) (7.5 g, 25 mmol) was dissolved in DMF (100 mL) and 3-iodoprop-1-ene (6.38 g, 38 mmol) was added. The mixture was cooled to 0° C. with vigorous stirring, then NaH (1.5 g of a 60% dispersion in mineral oil, 38 mmol) was added. The mixture was stirred for 30 min, and water (50 mL) was slowly added to quench the reaction. The aqueous mixture was extracted with EtOAc (300 mL) and the organic layer was washed with water (100 mL) and then with a brine solution (100 mL), and dried over anhydrous Na_2SO_4. After evaporation, 8.6 g of a yellow oily residue was isolated. This material was dissolved in Et_2O (100 mL), and concentrated hydrochloric acid (20 mL) was added at 0° C. with stirring. The mixture was then stirred for an additional 10 min. The mixture was extracted with EtOAc (200 mL) and 1N NaOH (200 mL). The organic layer was separated and washed with H_2O (150 mL) and with a brine solution (150 mL) and dried over anhydrous Na_2SO_4. The solvents were removed in vacuo and the resultant residue was purified by flash column chromatography (pet ether/EtOAc=10/1 to 5/1) to yield 2-(propen-2-yl)amino-4-chloro-7-methyl-pyrrolo[2,3-d]pyrimidine (6 g, 100% yield) as a yellow solid. LCMS: (ESI) m/z=223 $(M+H)^+$.

N-(2-(Propen-2-yl)amino-7-methyl-pyrrolo[2,3d] pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine (CLVIII)

To 2-(propen-2-yl)amino-4-chloro-7-methyl-pyrrolo[2,3-d]pyrimidine (CLVII, 500 mg, 2.38 mmol) were added n-butanol (5 mL), potassium carbonate (1.64 g, 5.0 eq.) and N,O-dimethylhydroxylamine hydrochloride (923 mg, 4.0 eq.). The mixture was stirred in an autoclave equipped with a stirrer at 100° C. for 8 h. After cooling to room temperature, water (20 mL) was added, and the mixture was extracted with EtOAc (3×20 mL). The combined organic layers were washed with water and with a brine solution and dried over anhydrous Na_2SO_4. The solvents were then removed in vacuo and the resultant residue was purified by flash column chromatography (pet ether/EtOAc=3/1) to yield N-(2-(propen-2-yl)amino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine (CLVIII, 310 mg, 55% yield).

N-(2-(Propen-2-yl)amino-7-methyl-pyrrolo[2,3d] pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine hydrochloride (CLIX)

N-(2-(Propen-2-yl)amino-7-methyl-pyrrolo[2,3 d]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine (310 mg, 1.32 mmol) was dissolved in H_2O (5 mL) and 0.5 M aqueous HCl solution (2.7 mL), and the solution was lyophilized to yield N-(2-(propen-2-yl)amino-7-methyl-pyrrolo[2,3 d]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine hydrochloride (CLIX, 325 mg) as a white solid. LCMS (ESI) m/z=248 $(M+H)^+$. ^1H NMR (500 MHz, DMSO) δ (ppm) 12.60-14.80 (br, 1H), 7.70-8.70 (br, 1H), 7.06 (s, 1H), 6.55 (s, 1H), 5.90-

5.94 (m, 1H), 5.31 (d, J=17.0 Hz, 1H), 5.17 (d, J=10.5 Hz, 1H), 4.06 (s, 2H), 3.84 (s, 3H), 3.70 (s, 3H), 3.53 (s, 3H).

Example 83

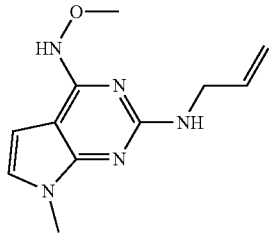
N-(2-(Propen-2-yl)amino-7-methyl-pyrrolo[2,3d] pyrimidin-4-yl)-O-methyl-hydroxylamine (CLX)

Example 84

N-(2-(Propen-2-yl)amino-7-methyl-pyrrolo[2,3d] pyrimidin-4-yl)-O-methyl-hydroxylamine hydrochloride (CLXI)

Scheme 55

CLX



N-(2-(Propen-2-yl)amino-7-methyl-pyrrolo[2,3d] pyrimidin-4-yl)-O-methyl-hydroxylamine (CLX)

2-(2-Propen-2-yl)amino-4-chloro-7-methyl-pyrrolo[2,3-d]pyrimidine (CLVII) (700 mg, 3.15 mmol) was dissolved in n-butanol, and potassium carbonate (2.2 g, 5.0 eq.) and O-methylhydroxylamine hydrochloride (768 mg, 4.0 eq.) were added. The mixture was stirred in an autoclave equipped with a stirrer at 100° C. for 12 h. After cooling to room temperature, water (20 mL) was added, and the mixture was extracted with EtOAc (3×25 mL). The combined organic layers were washed with water and a brine solution, and dried over anhydrous Na_2SO_4. The solvents were removed in vacuo and the resultant residue was purified by flash column chromatography (pet ether/EtOAc=5/1) to yield N-(2-(propen-2-yl) amino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-O-methyl-hydroxylamine (370 mg, 46% yield).

N-(2-(Propen-2-yl)amino-7-methyl-pyrrolo[2,3d] pyrimidin-4-yl)-O-methyl-hydroxylamine hydrochloride (CLXI)

N-(2-(propen-2-yl)amino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-O-methyl-hydroxylamine (370 mg, 1.59 mmol) was dissolved in H_2O (5 mL) and 0.5 M aqueous HCl solution (2 mL), and the solution was lyophilized to yield N-(2-(propen-2-yl)amino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-O-methyl-hydroxylamine hydrochloride (383 mg) as a white solid. LCMS (ESI) m/z=234 $(M+H)^+$. ^1H NMR (500 MHz, DMSO) δ (ppm) 12.40-13.00 (br, 1H), 7.70-8.10 (br, 1H), 7.04 (d, J=3.5 Hz, 1H), 6.49 (d, J=3.0 Hz, 1H), 5.94-5.99 (m, 1H), 5.32 (d, J=17.0 Hz, 1H), 5.17 (d, J=10.5 Hz, 1H), 4.04 (s, 2H), 3.85 (s, 3H), 3.61 (s, 3H).

Example 85

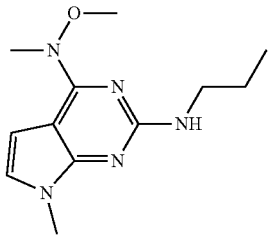
N-(2-n-Propylamino-7-methyl-pyrrolo[2,3 d]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine (CLXII)

Example 86

N-(2-n-Propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine hydrochloride (CLXIII)

Scheme 56

CLXII



N-(2-n-Propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-N,O-dimethyl-hydroxylamine (CLXII)

The desired compound was prepared from 4-chloro-2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidine and N,O-dimethyl-hydroxylamine as described in Example 73. LCMS: (ESI) m/z=250 $(M+H)^+$. ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.70 (s, 1H), 7.02 (s, 1H), 6.52 (s, 1H), 3.82 (s, 3H), 3.67 (s, 3H), 3.53 (s, 3H), 3.34 (t, J=8.5 Hz, 2H), 1.54-1.59 (m, 2H), 0.93 (t, J=8.5 Hz, 3H).

Example 87

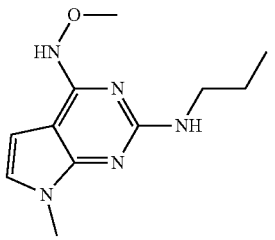
N-(2-n-Propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-O-methyl-hydroxylamine (CLXIV)

Example 88

N-(2-n-Propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-O-methyl-hydroxylamine hydrochloride (CLXV)

Scheme 57

CLXIV



N-(2-n-Propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-O-methyl-hydroxylamine (CLXIV)

The desired compound was prepared from 4-chloro-2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidine and O-methyl-hydroxylamine as described in Example 73. LCMS (ESI) m/z=236 $(M+H)^+$. ^1H NMR (500 MHz, DMSO) δ (ppm) 12.50 (s, 1H), 8.10 (s, 1H), 7.05 (s, 1H), 6.01 (s, 1H), 3.85 (s, 3H), 3.60 (s, 3H), 3.36 (s, 1H), 3.34 (t, J=6.5 Hz, 2H), 1.58-1.62 (m, 2H), 0.96 (t, J=7.5 Hz, 3H).

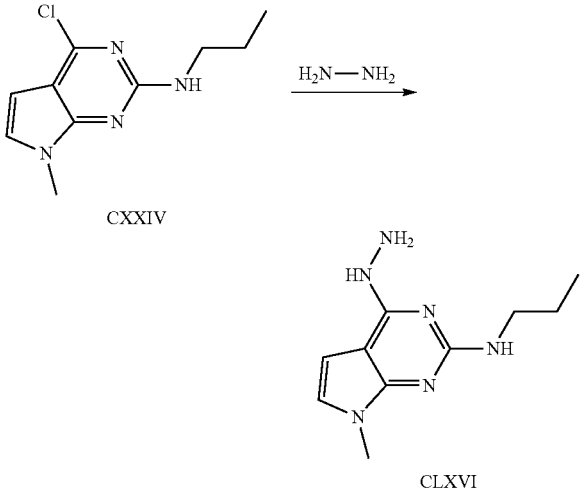
Example 89

N-(2-n-Propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine (CLXVI)

Example 90

N-(2-n-Propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine hydrochloride (CLXVII)

Scheme 58



N-(2-n-Propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine (CLXVI)

To a solution of 4-chloro-2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidine (CXXIV, 1.0 g, 4.5 mmol) in ethanol (70 mL) was added hydrazine (14 mL) and the mixture was heated at reflux for 3 h. After cooling to room temperature, the solvents were removed in vacuo. The residue was purified by flash column chromatography (CH_2Cl_2/MeOH=30/1) to yield N-(2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine (CLXVI, 1.0 g, 80% yield).

N-(2-n-Propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine hydrochloride (CLXVII)

N-(2-n-Propylamino-7-methyl-pyrrolo[2,3 d]pyrimidin-4-yl)-hydrazine (460 mg, 2.0 mmol) was dissolved in H_2O (10 mL) and 0.5 M aqueous HCl solution (4 mL), and the solution was lyophilized to yield N-(2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine hydrochloride (440 mg) as a brown solid. LCMS (ESI) m/z=221 $(M+H)^+$. ^1H NMR (500 MHz, DMSO) δ (ppm) 10.63 (s, 1H), 7.56 (s, 2H), 6.97 (d, J=3.0 Hz, 1H), 6.54 (d, J=3.5 Hz, 1H), 3.58 (s, 3H), 3.33 (s, 2H), 1.56-1.61 (m, 2H), 0.95 (t, J=8.0 Hz, 3H).

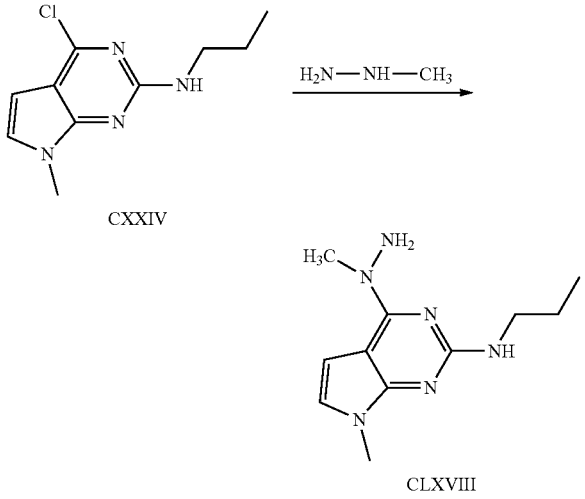
Example 91

N-Methyl-N-(2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine (CLXVIII)

Example 92

N-Methyl-N-(2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine hydrochloride (CLXIX)

Scheme 59



N-Methyl-N-(2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine (CLXVIII)

To a solution of 4-chloro-2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidine (CXXIV, 800 mg, 3.42 mmol) in n-butanol (5 mL) was added potassium carbonate (2.36 g, 5.0 eq.) and methylhydrazine (630 mg, 4.0 eq.). The mixture was stirred in an autoclave equipped with a stirrer at 100° C. for 15 h. After cooling to room temperature, water was added (20 mL), and the mixture was extracted with EtOAc (3×25 mL). The combined organic layers were washed with water and with a brine solution, and dried over anhydrous Na_2SO_4. The solvents were removed in vacuo and the resultant residue was purified by flash column chromatography (pet ether/EtOAc=3/1 to DCM/MeOH=10/1) to yield N-methyl-N-(2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine (CLXVIII, 220 mg, 36% yield).

N-Methyl-N-(2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine hydrochloride (CLXIX)

N-methyl-N-(2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine (220 mg, 0.94 mmol) was dissolved in H_2O (10 mL) and 0.5 M aqueous HCl solution (2 mL), and the solution was lyophilized to yield N-methyl-N-

(2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine hydrochloride (CLXIX, 238 mg) as a brown solid. LCMS (ESI) m/z=235 $(M+H)^+$. ^1H NMR (500 MHz, DMSO) δ (ppm) 7.50-8.10 (br, 4H), 7.05 (s, 1H), 6.67 (s, 1H), 3.60 (s, 3H), 3.58 (s, 3H), 3.33 (s, 2H), 1.55-1.62 (m, 2H), 0.95 (t, J=7.5 Hz, 3H).

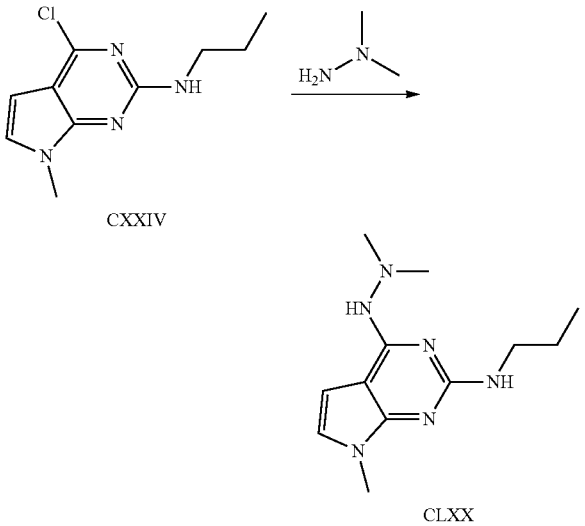
Example 93

N,N-Dimethyl-N'-(2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine (CLXX)

Example 94

N,N-Dimethyl-N'-(2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine hydrochloride (CLXXI)

Scheme 60



N,N-Dimethyl-N'-(2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine (CLXX)

To a solution of 4-chloro-2-n-propylamino-7-methyl-pyrrolo[2,3d]-pyrimidine (1.1 g, 5 mmol) and 1,1-dimethylhydrazine (450 mg, 7.5 mmol) in dioxane (30 mL) was added xphos (622 mg, 1 mmol), $Pd_2(dba)_3$ (458 mg, 0.5 mmol) and Cs_2CO_3 (2.45 g, 7.5 mmo). The solution was degassed by bubbling argon through it for 10 min using a syringe needle. The mixture was then stirred at 80° C. for 2 h. After cooling to room temperature, water was added (20 mL) and the mixture was extracted with EtOAc (3×40 mL). The combined organic layers were washed with water and with a brine solution, and dried over anhydrous Na_2SO_4. The solvents were removed in vacuo and the resultant residue was purified by flash column chromatography (DCM/MeOH=30/1 to 5/1) to yield N,N-dimethyl-N'-(2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine (CLXX, 100 mg, 8% yield, ~85% purity).

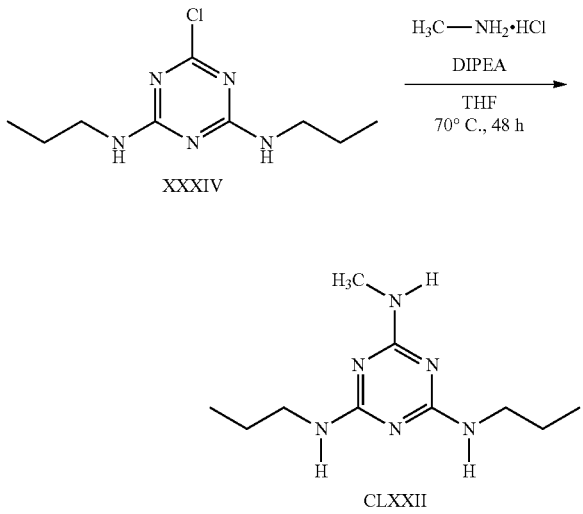
N,N-Dimethyl-N'-(2-n-propylamino-7-methyl-pyrrolo[2,3d]pyrimidin-4-yl)-hydrazine hydrochloride (CLXXI)

N,N-dimethyl-N'-(2-n-propylamino-7-methyl-pyrrolo [2,3 d]pyrimidin-4-yl)-hydrazine (100 mg, 0.4 mmol) was dissolved in H_2O (5 mL) and 0.5 M aqueous HCl solution (1 mL) and the solution was lyophilized to yield N,N-dimethyl-N'-(2-n-propylamino-7-methyl-pyrrolo[2,3-d]pyrimidin-4-yl)-hydrazine hydrochloride (110 mg) as a yellow oil. LCMS (ESI) m/z=249 $(M+H)^+$. ^1H NMR (500 MHz, MeOD) δ (ppm) 6.92 (d, J=4.0 Hz, 1H), 6.50 (d, J=3.0 Hz, 1H), 3.66 (s, 3H), 3.45 (t, J=7.5 Hz, 2H), 2.79 (s, 6H), 1.67-1.72 (m, 2H), 1.01-1.04 (m, 3H).

Example 95

N-(2,4-Bis-n-propylamino)-N-(6-methylamino)-[1,3,5]triazine (CLXXII))

Scheme 61



A pressure tube was charged with 2-chloro-N-(4,6-bis-(n-propylamino)-[1,3,5-triazine (XXXIV) (1.50 g, 6.53 mmol), N,N-diisopropylethylamine (5.4 mL, 32.65 mmol), methylamine hydrochloride (2.2 g, 32.65 mmol) and tetrahydrofuran (20 mL). The reaction mixture was heated at 70° C. for 48 h. An additional amount of N,N-diisopropylethylamine (5.4 mL, 32.65 mmol) and methylamine hydrochloride (2.2 g, 32.65 mmol) were added, and the reaction mixture was heated at 70° C. for an additional 48 h. The mixture was poured into saturated $NaHCO_3$ solution (40 mL). The suspension was extracted with EtOAc (3×70 mL). The combined organic extracts were washed with water (70 mL), then with a brine solution (70 mL) and lastly dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography using gradient elution from petroleum ether/EtOAc (9:1, (v/v)) to petroleum ether/EtOAc (1:4, (v/v)) to yield N-(2,4-bis-n-propylamino)-N-(6-methylamino)-[1,3,5]triazine (0.35 g, 24%). 400 MHz ^1H NMR (DMSO-d_6, ppm) 6.69-

6.02 (3H, m), 3.22-3.02 (4H, m), 2.68 (3H, s), 1.55-1.38 (4H, m), 0.84 (6H, t, J=7.4 Hz). ESI-MS (m/z): 225 $[M+H]^+$.

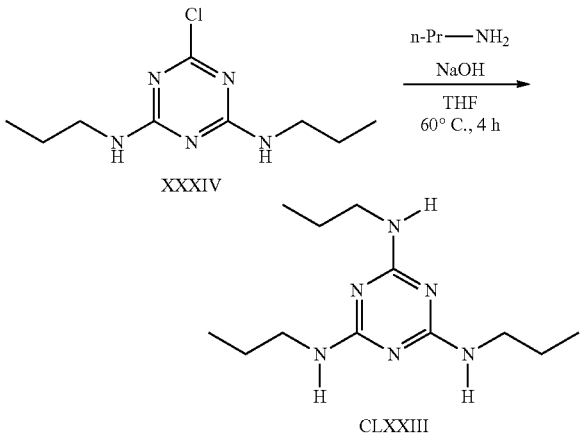
Example 96

N-(2,4,6-Tris-n-propylamino)-[1,3,5]triazine (CLXXIII)

Example 97

N-(2,4,6-Tris-n-propylamino)-[1,3,5]triazine hydrogen sulfate (CLXXIV)

Scheme 62



N-(2,4,6-Tris-n-propylamino)-[1,3,5]triazine (CLXXIII)

A mixture of 2-chloro-N-(4,6-bis-(n-propylamino)-[1,3,5-triazine (XXXIV) (1.00 g, 4.35 mmol), NaOH (348 mg, 8.70 mmol) and n-propylamine (1.4 mL, 17.40 mmol) in 1,4-dioxane (40 mL) was heated at 60° C. for 4 h. The volatiles were then removed under reduced pressure. Water (50 mL) was added to the residue and the mixture was extracted with EtOAc (3×50 mL). The combined organic extracts were washed with water (50 mL), then with a brine solution (50 mL) and lastly, dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography using gradient elution from CH_2Cl_2/EtOH (99:1, (v/v)) to CH_2Cl_2/EtOH (97:3 (v/v)) to yield N-(2,4,6-tris-n-propylamino)-[1,3,5]triazine (790 mg, 72%). 400 MHz ^{1}H-NMR ($CDCl_3$, ppm) 4.93-4.51 (3H, br s), 3.41-3.22 (6H, m), 1.62-1.49 (6H, m), 0.94 (9H, t, J=7.4 Hz). ESI-MS (m/z): 253 $[M+H]^+$.

N-(2,4,6-Tris-n-propylamino)-[1,3,5]triazine hydrogen sulfate (CLXXIV)

The title compound was prepared from N-(2,4,6-tris-n-propylamino)-[1,3,5]triazine and 95% H_2SO_4 in quantitative yield, as described in Example 8A. 400 MHz ^{1}H-NMR (DMSO-d6, ppm) 8.26 (1H, br s) 8.10-7.99 (1H, m) 7.94-7.85 (1H, m) 3.33-3.16 (6H, m) 1.60-1.45 (6H, m) 0.88 (9H, t, J=7.5 Hz). ESI-MS (m/z): 253 $[M+H]^+$.

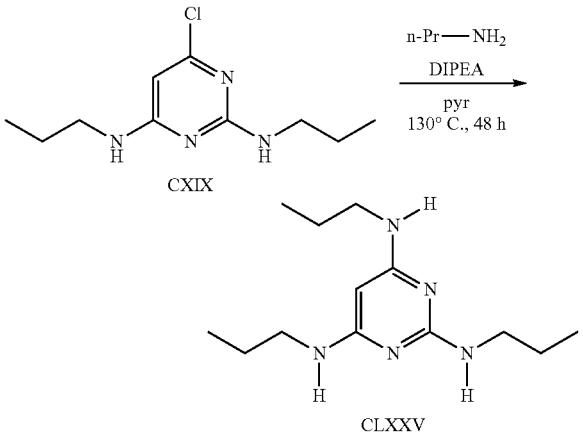
Example 98

N-(2,4,6-Tris-n-propylamino)-1,3-pyrimidine (CLXXV)

Example 99

N-(2,4,6-Tris-n-propylamino)-1,3-pyrimidine hydrogen sulfate (CLXXVI)

Scheme 63



N-(2,4,6-Tris-n-propylamino)-4,3-pyrimidine (CLXXV)

A pressure tube was charged with 4-chloro-2,6-bis-(N-n-propylamino)-1,3-pyrimidine (CXIX) (1.53 g, 6.65 mmol), n-propylamine (2.7 mL, 33.25 mmol), N,N-diisopropylethylamine (2.3 mL, 13.30 mmol) and pyridine (15 mL). The reaction mixture was heated at 130° C. for 48 hours. The volatiles were removed under reduced pressure. Water (50 mL) was added to the residue and the mixture was extracted with CH_2Cl_2 (3×50 mL). The combined organic extracts were washed with water (100 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography using gradient elution from CH_2Cl_2/EtOH (99:1, (v/v)) to CH_2Cl_2/EtOH (95:5, (v/v)) to yield N-(2,4,6-tris-n-propylamino)-1,3-pyrimidine (0.90 g, 54%). 400 MHz ^{1}H NMR (DMSO-d_6, ppm) 5.43 (1H, br s), 5.15 (1H, br s), 4.74 (1H, s), 3.27 (2H, td, J=6.9, 5.9 Hz), 3.16-3.09 (4H, m), 1.65-1.51 (6H, m), 0.96 (6H, t, J=7.4 Hz), 0.93 (3H, t, J=7.3 Hz). ESI-MS (m/z): 252 $[M+H]^+$.

N-(2,4,6-Tris-n-propylamino)-4,3-pyrimidine hydrogen sulfate (CLXXVI)

The title compound was prepared from N-(2,4,6-tris-n-propylamino)-1,3-pyrimidine and 95% H_2SO_4 in quantitative yield, as described in Example 8A. 400 MHz ^{1}H-NMR (DMSO-d6, ppm) 10.81-10.58 (1H, br s) 7.9-7.7 (1H, m)

7.6-6.5 (3H, m) 5.01 (1H, s) 3.30-3.12 (4H, m) 3.09-2.94 (2H, m) 1.59-1.46 (6H, m) 0.94-0.84 (9H, m). ESI-MS (m/z): 252 $[M+H]^+$.

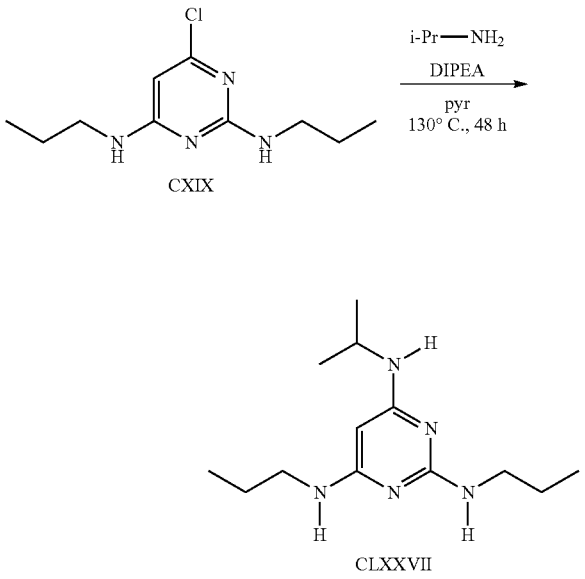
Example 100

N-(4-i-Propylamino)-N-(2,6-bis-n-propylamino)-1,3-pyrimidine (CLXXVII)

Example 101

N-(4-i-Propylamino)-N-(2,6-bis-n-propylamino)-1,3-pyrimidine hydrogen sulfate (CLXXVIII)

Scheme 64



N-(4-i-Propylamino)-N-(2,6-bis-n-propylamino)-1,3-pyrimidine (CLXXVII)

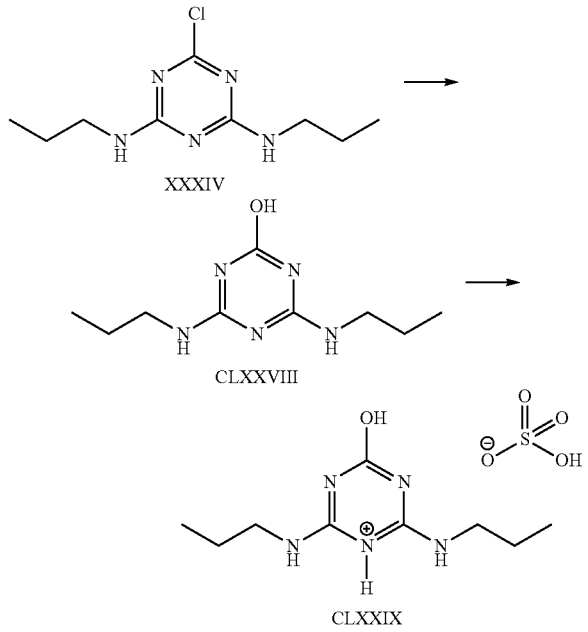
The title compound was prepared from 4-chloro-2,6-bis-(N-n-propylamino)-1,3-pyrimidine (CXIX) and i-propylamine as described in Example 98 in 16% yield. 400 MHz ^{1}H NMR (DMSO-d6, ppm) 5.78 (1H, br s), 5.46 (1H, br s), 5.19 (1H, br s), 4.73 (1H, s), 3.83-3.71 (1H, m), 3.34-3.27 (2H, m), 3.19-3.10 (2H, m), 1.68-1.53 (4H, m), 1.23 (6H, d, J=6.4 Hz), 0.98 (3H, t, J=7.4 Hz), 0.94 (3H, t, J=7.4 Hz). ESI-MS (m/z): 252 $[M+H]^+$.

N-(4-i-Propylamino)-N-(2,6-bis-n-propylamino)-1,3-pyrimidine hydrogen sulfate (CLXXVIII)

The title compound was prepared from N-(4-i-propylamino)-N-(2,6-bis-n-propylamino)-1,3-pyrimidine and 95% H_2SO_4 in quantitative yield, as described in Example 8A. 400 MHz ^{1}H-NMR (DMSO-d_6, ppm) 0.76-10.40 (1H, m), 8.0-7.1 (3H, m), 5.01 (1H, s), 4.18-3.93 (1H, m), 3.35-3.13 (3H, m), 3.12-2.94 (1H, m), 1.60-1.45 (4H, m), 1.19-1.11 (6H, m), 0.93-0.83 (6H, m). ESI-MS (m/z): 252 $[M+H]^+$.

Comparative Example 1

Scheme 65



N-(4,6-Bis(propylamino)-1,3,5-triazin-2-ol (CLXX-VIII)

A vial was charged with 6-chloro-N^2,N^4-dipropyl-1,3,5-triazine-2,4-diamine (XXXIV) (800 mg, 3.48 mmol), NaOH (696 mg, 17.40 mmol), water (3 mL) and 1,4-dioxane (3 mL). The reaction mixture was heated at 140° C. for 30 min under microwave irradiation. The mixture was acidified with 1N HCl to pH 5, precipitate were filtered, washed with water. The product was dissolved in 50% trifluoroacetic acid (30 mL), the indissoluble residue was filtered off. The filtrate was neutralized with saturated $NaHCO_3$ solution, the formed precipitate was filtered, washed with water, acetone and dried to give 550 mg (75%) of 4,6-bis(propylamino)-1,3,5-triazin-2-ol (CLXXVIII). 400 MHz ^{1}H NMR (DMSO-d_6+CF_3COOD, ppm) 3.18-3.34 (4H, m), 1.45-1.59 (4H, m), 0.86 (6H, t). ESI-MS (m/z): 212 $[M+H]^+$.

N-(4,6-Bis(propylamino)-1,3,5-triazin-2-ol hydrogen sulfate (CLXXIX)

95% H_2SO_4 (28 μL, 0.52 mmol) was added to the suspension of 4,6-bis(propylamino)-1,3,5-triazin-2-ol (CLXXVIII) (110 mg, 0.52 mmol) in 1,4-dioxane (2 mL). The mixture was stirred for 30 min at room temperature. The resultant precipitate were filtered, washed with Et_2O and dried to afford 4,6-bis(propylamino)-1,3,5-triazin-2-ol hydrogen sulfate (CLXXIX) in quantitative yield. 400 MHz ^{1}H NMR (CD_3OD+CF_3COOD, ppm) 3.35-3.45 (3.2H, m), 3.22-3.27 (0.8H, m), 1.58-1.70 (4H, m), 0.91-1.0 (6H, m). ESI-MS (m/z): 212 $[M+H]^+$. Major Fragments (M+H) 170, 128, 86.

Example 102

Preparation of (XXXVI) Salt Form A (FIG. **1**)

Method A:

To (XXXV) in ethyl acetate at ambient temperature (ca. 151 mg/mL, clear solution) was added a solution of one molar equivalent of concentrated sulfuric acid in ethanol. The clear solution was stirred at ambient temperature for ca. 1 day (clear), after which 6 volume equivalents of isopropyl ether (vs. ethyl acetate) were added, resulting in a cloudy mixture. The mixture was stirred for 3 days, then filtered to yield solid product (Hydrogen Sulfate Salt Form A).

Method B:

To (XXXV) in methyl ethyl ketone at ca. 65° C. (ca. 104 mg/mL, clear solution) was added one molar equivalent concentrated sulfuric acid. A precipitate that immediately formed rapidly redissolved. The mixture was stirred at ca. 65° C. for ca. 5 h and slowly cooled over ca. 1 h to ambient temperature. The mixture was stirred for one day at ambient temperature, then filtered to yield sold product (61% yield) (Hydrogen Sulfate Salt Form A).

Method C:

To (XXXV) in methyl ethyl ketone at ca. 65° C. (ca. 100 mg/mL, clear solution) was added one molar equivalent of concentrated sulfuric acid. A precipitate that formed immediately rapidly redissolved. The mixture was stirred at ca. 65° C. for ca. 1 h and slowly cooled over 1 h to ambient temperature, yielding solids in the form of rods. The mixture was stirred for ca. 4 h at ambient temperature. One volume equivalent of heptane (vs. methyl ethyl ketone) was added and the mixture was stirred for 1 day at ambient temperature, then was filtered to collect solid product (81% yield) (Hydrogen Sulfate Salt Form A).

Method D:

To (XXXV) in methyl ethyl ketone at ca. 69° C. (ca. 200 mg/mL, clear solution) was added one molar equivalent of concentrated sulfuric acid. A precipitate that formed immediately rapidly redissolved. The mixture was stirred at ca. 69° C. for ca. 1 h and quickly cooled to ambient temperature and stirred for ca. 0.5 h, yielding a thick paste. The mixture was stirred in an ice bath for ca. 45 minutes, then filtered to collect solid product (Hydrogen Sulfate Salt Form A).

Method E:

To (XXXV) in methyl ethyl ketone at ca. 67° C. (ca. 101 mg/mL, clear solution) was added one molar equivalent of concentrated sulfuric acid. The mixture was stirred at ca. 67° C. for ca. 1 h and slowly cooled to ambient temperature. The mixture was stirred for ca. 1.5 h at ambient temperature, then one volume equivalent (vs. methyl ethyl ketone) of methyl tert-butyl ether was added, the mixture was stirred for one day, then filtered to collect solid product (76% yield) (Hydrogen Sulfate Salt Form A).

Method F:

To (XXXV) in acetone at ca. 50° C. (ca. 199 mg/mL, clear solution) was added one molar equivalent of concentrated sulfuric acid, forming a clear solution. The mixture was stirred at ca. 50° C. for ca. 1 h and slowly cooled to ambient temperature, at which point solids had formed. To the mixture was added about two volume equivalents (vs. methyl ethyl ketone) of methyl tert-butyl ether. The mixture was stirred for one day at ambient temperature, then filtered to collect solid product (79% yield) (Hydrogen Sulfate Salt Form A).

Example 103

Preparation of a Mixture of (XXXVI) Salt Form A and (XXXVI) Salt Form B

To (XXXV) in ethyl acetate at ca. 65° C. (99 mg/mL, clear solution) was added one molar equivalent of concentrated sulfuric acid. A thick suspension within 5 minutes along with a small amount of tacky material. The mixture was stirred at ca. 65° C. for about 5 h, slowly cooled to ambient temperature, stirred for 4 days then filtered to collect solid product (Hydrogen Sulfate Salt Form A as a Mixture with Form B), FIG. **23**

Example 104

Preparation of (XXXVI) Salt Form C

A sample of (XXXVI) Form A was exposed to 97% relative humidity at ambient temperature for 4 days. The resulting solid form was designated Hydrogen Sulfate Form C. The resulting material under thermal gravimetric testing lost 4.6 wt % between about 30 and 50° C., consistent with approximately one molar equivalent of water (FIG. **24**).

Example 105

Preparation of (XXXVI) Salt Form D

To (XXXV) in ethyl acetate at ca. 65° C. (ca. 100 mg/mL) was added one molar equivalent of concentrated sulfuric acid. Tacky material immediately precipitated and more solids were observed after ca. 5 minutes. A subsample of these solids were removed with a pipette and filtered without cooling. These solid was designated Hydrogen Sulfate Salt Form D (FIG. **25**).

Example 106

Preparation of a Mixture of (XXXVI) Salt Form A and (XXXVI) Salt Form D

To (XXXV) in isopropyl acetate at ca. 80° C. (203 mg/mL, clear solution) was added one molar equivalent of concentrated sulfuric acid as a solution in isopropyl acetate. A precipitate formed. The mixture was stirred at ca. 80° C. for ca. 45 minutes, then was slowly cooled to ambient temperature, stirred for day, then filtered to yield the solid product (83% yield); Hydrogen Sulfate Form A in a Mixture with Hydrogen Sulfate Form D (FIG. **26**).

Example 107

Preparation of N-(4,6-Bis-n-propylamino-[1,3,5] triazin-2-yl)-N,O-dimethyl-hydroxylamine Phosphoric Acid Salt (CLXXX), Form A

To (XXXV) in acetonitrile at ambient temperature were added two molar equivalents of phosphoric acid. The mixture was stirred for 2 days, then the solid that was formed was collected by filtration and dried on the filter under a nitrogen stream (Phosphoric Acid Salt Form A; FIG. **27**).

Example 108

Preparation of (CLXXX) Form C

To (XXXV) in methanol at ambient temperature (clear solution) was added one molar equivalent of phosphoric acid,

resulting in a clear solution. The vial containing the solution was placed in an enclosed chamber with a vial of isopropyl ether and vapor diffused, forming a solid which was collected by filtration (Phosphoric Acid Salt Form C, FIG. **28**).

Example 109

Preparation of a Mixture of (CLXXX) Form A and (CLXXX) Form B

To (XXXV) in acetonitrile (slurry) at ambient temperature was added one molar equivalent of phosphoric acid. A thick slurry formed. The sturry was sonicated at ambient temperature for 3 days, then was filtered to collect the solid product (Phosphoric Acid Salt Form A in a Mixture with Form B, FIG. **29**).

Example 110

Preparation of a Mixture of (CLXXX) Form C and (CLXXX) Form D

To (XXXV) in ethyl acetate (clear solution) were added two molar equivalents of phosphoric acid as a solution in ethanol. A precipitate formed. The mixture was further diluted with ethyl acetate, stirred for 3 days, then filtered to collect solid product (a mixture of Phosphoric Acid Salt Form C and Phosphoric Acid Salt Form D, FIG. **30**).

Example 111

Preparation of a Mixture of (CLXXX) Form C and (CLXXX) Form E

To (XXXV) in tetrahydrofuran (clear solution) at ambient temperature were added three molar equivalents of phosphoric acid. A precipitate formed. The mixture was stirred at ambient temperature for 3 days, then filtered to collect solid product (a mixture of Phosphoric Acid Salt Form C and Phosphoric Acid Salt Form E, FIG. **31**).

Example 112

Effect of Compound (XXXVI) on Opioid-Induced Respiratory Depression in the Rat

Rats with pre-cannulated jugular vein (for administering drugs) were acclimated to plethysmography chambers for a minimum of 60 minutes, or until animals were no longer restless. Each animal was dosed with morphine sulfate (10 mg/kg), dissolved in sterile water at a concentration of 10 mg/mL (supplied by Baxter Healthcare Corporation), via injection into the jugular vein catheter over a period of 5-10 seconds.

Compound (XXXVI) was dissolved in 20% hydroxypropyl β-cyclodextran (20% bcd on graph) at a concentration of 0.45 mg/mL at pH 5. After a period of 5 min, compound (XXXVI), labeled as cmpd (A), was administered via infusion into the jugular vein at a dose of 0.10 mg/kg/min for 20 min and then at a dose of 0.30 mg/kg/min for 20 min (e.g., 20 μL/min/0.3 kg rat to yield 0.03 mpk/min).

After 20 minutes of infusion at this dose, the infusion pumps were turned off, and all animals were given a 20 minute recovery period, followed by a post-study analysis of rat health and behavior. The minute ventilation data indicate that compound (XXXVI) significantly reversed opioid-induced respiratory depression in rat compared to vehicle. Results are illustrated in FIG. **1**.

Example 113

Effects of Morphine and Compound (XXXVI) on Blood Gases in the Rat

Rats with pre-cannulated jugular vein and femoral arterial catheters (for administering drugs and obtaining blood samples respectively) were obtained from Harlan laboratories and kept at the animal facility at Galleon Pharmaceuticals until the experimental procedures. Each animal was dosed with morphine sulfate (10 mg/kg), dissolved in saline at a concentration of 10 mg/mL, via injection into the jugular vein over a period of 20 seconds with a 20 second flush of 0.9% NaCl saline. Prior to morphine administration, two 250 μL samples of arterial blood were aspirated from the femoral artery into a pre-heparinized syringe. The samples were analyzed on Radiometer's ABL Flex 800, where pO_2, pCO_2, pH, SaO_2 and other parameters were recorded. Aspirated volumes of arterial blood were replaced by room temperature sterile saline (~300 μL) slowly flushed back into the femoral arterial catheter of the rodent to prevent hypovolemia. Morphine was then administered and 2 minutes later another blood sample was taken.

After a period of 5 min from the administration of morphine, compound (XXXVI), labeled as cmpd (A), was administered via infusion into the jugular vein at a dose of 0.1, 0.3 and 1.0 mg/kg/min (dissolved in PBS buffer). The infusion started at t=15 minutes and ended at t=35 minutes. Arterial blood gas analysis occurred at time points t=12, 18, 25, 35, 40, 45, and 50 minutes. The data show that compound (XXXVI) significantly reverses opioid-induced respiratory depression in rat compared to vehicle. Results are illustrated in FIG. **2***a* and FIG. **2***b* and the accompanying Table 6.

TABLE 6

Cmpd	n	Dose (mg/kg/min)	pH (% reversal)	$PaCO_2$ (% reversal)	PaO_2 (% reversal)	SaO_2 (% reversal)
XXXVI	6	1	60	94	26	62
XXXVI	11	0.3	55	72	−16	43
XXXVI	10	0.1	37	47	16	51
XXXVI	6	0.03	20	18	−2.0	42

Example 114

Hypoxic Ventilatory Response (HVR) and Effect of Compound (XXXVI) on HVR in the Rat

Rats with a pre-cannulated jugular vein (for administering drugs) were acclimated to plethysmography chambers for a minimum of 60 minutes, or until animals were no longer restless. Each animal was dosed with compound (XXXVI), labeled as cmpd (A), at 0.03 mg/kg/min via infusion into the jugular vein catheter for a period of 50 minutes. After a period of 20 minutes, an isocapnic hypoxic mixture (12% O_2 balanced N_2) was administered into all chambers using a gas mixer (CWE inc. GSM-3 gas mixer) for 20 minutes. After this time, the gas mixer was turned off, resulting in normal room air pumped into the chambers. Ten minutes later, the infusion pumps were turned off, and all animals were given a 20 minute recovery period, followed by a post-study analysis of rat health and behavior. The minute ventilation data show that

compound (XXXVI) significantly potentiates the hypoxic ventilatory response in the rat compared to vehicle. Results are illustrated in FIG. **3**.

Example 115

Effect of Compound (XXXVI) on Opioid-Induced Respiratory Depression in the Monkey

Juvenile macaques (four-year-old *Macaca fascicularis*, 2 to 5 kg, n=13) were used for the study. Animal husbandry was conducted under USDA guidelines and the protocols were approved by the Institutional Animal Care and Use Committee of East Carolina University.

Anesthesia was induced with 5% isoflurane and then maintained with 1.5 to 2% isoflurane (100% O_2, 2 L/min). Antebrachial veins were cannulated. Vivometrics Lifeshirts (Ventura, Calif.) were fitted to the animals for monitoring respiratory function by inductance plethysmography. Abdomen and rib cage deflections were calibrated using the Qualitative Diagnostic Calibration (QDC) procedure, and tidal volume was normalized to 15 mL, which represented a typical tidal volume for these animals as previously measured using conventional techniques. Heart rate was monitored continuously by 3-lead ECG.

$ETCO_2$ was measured via a neonatal nasal cannula connected to a microstream CO_2 sensor (Cardell monitor, Model 9405). SpO_2 and HR were monitored by pulse oximetry using a reflectance probe (Nelcor Max-Fast) positioned on the inner aspect of the upper arm. HR was also determined from the ECG allowing measurement when animal activity compromised the integrity of pulse oximetry signals. BP was measured in anesthetized animals using a cuff positioned on the ankle.

Compound (XXXVI) was dissolved in 20% hydroxypropyl β-cyclodextran (HPBCD) and sterile filtered using a 2 μl syringe filter. Compound (XXXVI), labeled as cmpd (A), was then delivered at a rate of 0.20 mg/kg/min for 5 minutes, followed by reduction of the infusion rate to 0.10 mg/kg/min for 10 minutes. Minute Ventilation and end-tidal CO_2 were monitored. Naloxone HCl (0.05 mg/kg intravenous) was delivered to reverse morphine effects and conclude the experiment. The data showed that cmpd (A) produced a full reversal of end-tidal carbon dioxide increases caused by the opioid, and also increased minute ventilation (FIG. **4**).

Example 116

Dose-Dependent Effect of Compound (XXXVI) and (L) on Minute Ventilation (MV) in Naive Rats

All surgical procedures were performed under anesthesia induced by 2% isoflurane in compressed medical grade air. With rats in supine position, the right femoral vein was catheterized using polyethylene tubing (PE-50). This catheter was used for fluid and drug administration. Simultaneously, the right femoral artery was also catheterized for monitoring blood pressure. In order to measure the respiratory parameters in spontaneously breathing rats, trachea was intubated using 13 gauge tracheal tube (2.5 mm ID, Instech Solomon, Pa.).

After establishing a stable base-line at 1.5% isoflurane, cumulative dose-dependent (0.01, 0.03, 0.1, 0.3, 1, 3, 10 mg/kg) ventilatory responses to compounds (XXXVI) and (L), labeled as cmpd (A) and cmpd (B) respectively, were generated from spontaneously breathing rats. Maximum peak minute ventilatory (MV) values at each dose of corresponding drug were calculated and used for generating ED_{50} values. The results are shown in FIG. **5**. Both compounds (XXXVI) and (L) increased minute ventilation in a dose-dependent manner with calculated ED_{50} values of 0.14 and 0.13 mg/kg respectively.

Example 117

Effect of Compound (CXXI) on Dose-Dependent Minute Ventilation (MV) in Naive Rats

Compound (CXXI), labeled as cmpd (C), was shown to increase minute ventilation in a dose-dependent manner following the procedure above. Results are shown in FIG. **6**.

Example 118

Effects of Compound (L) in the Opioid-Treated Rat

Following the procedure in Example 6, Compound (L), labeled as cmpd (B), was shown to reverse the effects of opioid on blood gases and pH in the rat by increasing pH, SaO_2 and pO_2 and by decreasing pCO_2 levels, as illustrated in FIGS. **7**A-**7**D.

Example 119

Effects of Compound (CXLII) in the Opioid-Treated Rat

Following the procedure in Example 6, Compound (CXLII), labeled as cmpd (D), was shown to reverse the effects of opioid on blood gases and pH in the rat by increasing pO_2 levels, increasing (SaO_2) oxygen saturation, decreasing pCO_2 levels and raising pH, as illustrated in FIGS. **8**A-**8**D.

Example 120

Effects of Compound (CXLII) on Opioid-Induced Respiratory Depression in the Rat

Following the procedure in Example 5, Compound (CXLII), labeled as cmpd (D), was shown to reverse opioid-induced respiratory depression in the rat by increasing minute ventilation (MV) as determined by plethysmography. The results are illustrated in FIG. **9**.

Example 121

Effect of Compounds on Minute Ventilation (MV) and Cardiovascular Parameters in Naive Rats

All surgical procedures were performed under anesthesia induced by 2% isoflurane in compressed medical grade air. With rats in supine position, the right femoral vein was catheterized using polyethylene tubing (PE-50). This catheter was used for fluid and drug administration. Simultaneously, the right femoral artery was also catheterized for monitoring blood pressure and heart rate. In order to measure the respiratory parameters in spontaneously breathing rats, trachea was intubated using 13 gauge tracheal tube (2.5 mm ID, Instech Solomon, Pa.).

After establishing a stable base-line at 1.5% isoflurane, compounds (typically at a dose of 1 mpk) were administered IV, and ventilatory parameters were generated from spontaneously breathing rats, along with cardiovascular output (mean arterial pressure (MAP) and heart rate). Maximum peak minute ventilatory (MV) responses (MPR), along with changes in minute ventilation versus baseline (AMV) were obtained as shown in the tables below.

TABLE 7

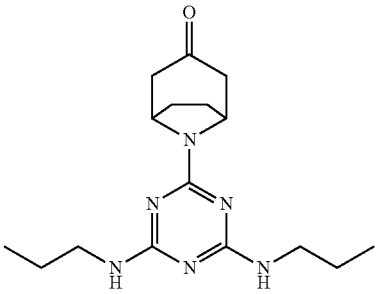
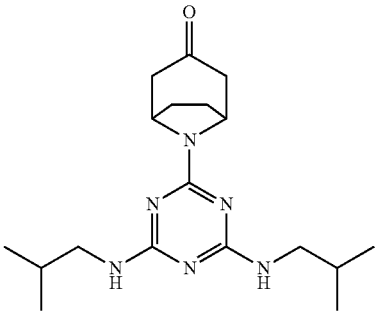
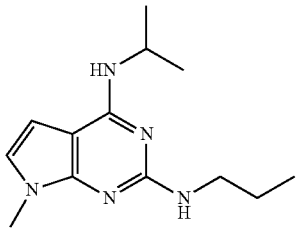
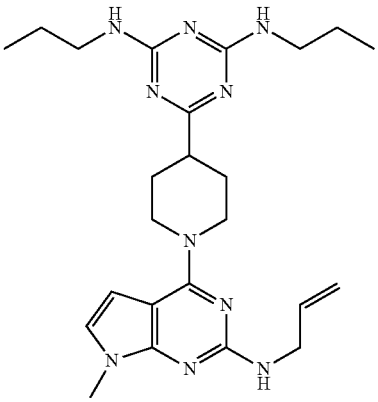
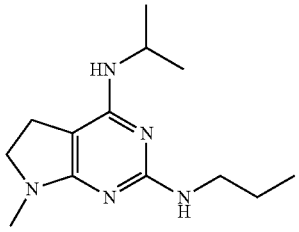
Cmpd	Structure	Formulation	Dose	pH	MV		CV	
					MPR	ΔMV	MAP (mm Hg)	Heart Rate (B/min)
(1,5)-8-(4,6-bis(n-propylamino)-1,3,5-triazin-2-yl)-8-azabicyclo[3.2.1]octan-3-one		20% HPβCD	1 mpk	5	213	98	114	308
(1,5)-8-(4,6-bis(isobutylamino)-1,3,5-triazin-2-yl)-8-azabicyclo[3.2.1]octan-3-one		20% HPβCD	1 mpk	5	237	137	117	345
N^4-isopropyl-7-methyl-N^2-n-propyl-7H-pyrrolo[2,3-d]pyrimidine-2,4-diamine		20% HPβCD	1 mpk	5	402	320	118	340
6-(1-(2-(allylamino)-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperidin-4-yl)-N^2,N^4-di-n-propyl-1,3,5-triazine-2,4-diamine		20% HPβCD	1 mpk	5	154	60	114	350
N^4-isopropyl-7-methyl-N^2-n-propyl-6,7-dihydro-5H-pyrrolo[2,3-d]pyrimidine-2,4-diamine		20% HPβCD	1 mpk	5	327	224	93	318

TABLE 7-continued

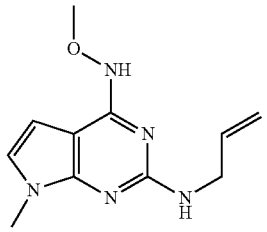
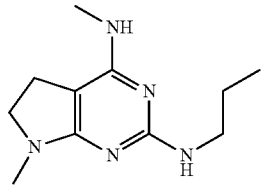
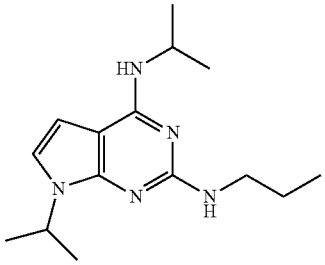
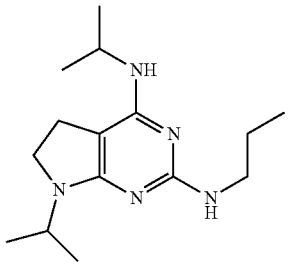
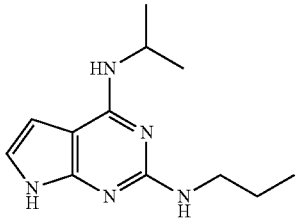
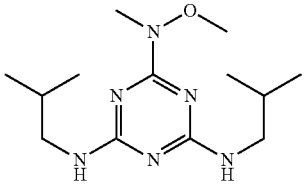
Cmpd	Structure	Formulation	Dose	pH	MV MPR	MV ΔMV	CV MAP (mm Hg)	CV Heart Rate (B/min)
CLX, CLXI		20% HPβCD	1 mpk	5	182	103	96	336
CXXXI, CXXXII		20% HPβCD	1 mpk	4	204	130	101	348
N^4,7-diisopropyl-N^2-n-propyl-7H-pyrrolo[2,3-d] pyrimidine-2,4-diamine		20% HPβCD	1 mpk	6	250	155	103	356
CXXXVI, CXXXVII		20% HPβCD	1 mpk	4	238	143	98	389
N^4-isopropyl-N^2-n-propyl-7H-pyrrolo[2,3-d] pyrimidine-2,4-diamine		20% HPβCD	1 mpk	4	259	172	96	387
XXIX		20% HPβCD	1 mpk	4-6	253	161	92	371

TABLE 7-continued

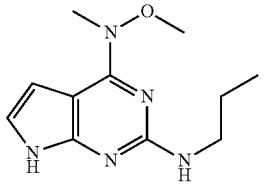
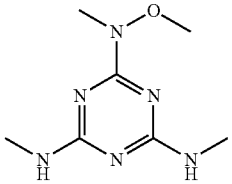
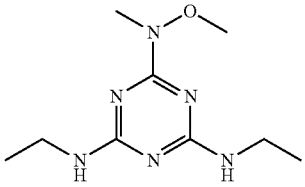
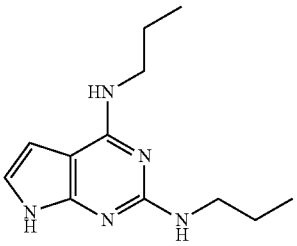
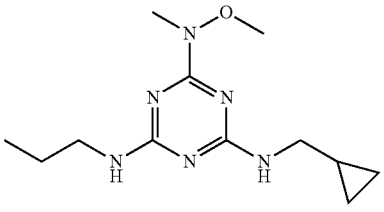
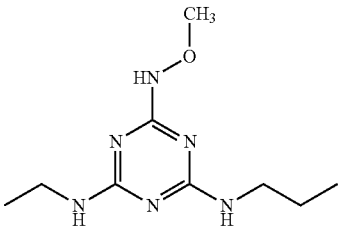
Cmpd	Structure	Formulation	Dose	pH	MV MPR	MV ΔMV	CV MAP (mm Hg)	CV Heart Rate (B/min)
CXLI, CXLII		20% HPβCD	1 mpk	4-6	264	163	93	336
XX		20% HPβCD	1 mpk	4-6	213	60	92	432
XXII		20% HPβCD	1 mpk	4-6	252	130	95	415
N^2,N^4-di-I-propyl-7H-pyrrolo[2,3-d] pyrimidine-2,4-diamine		20% HPβCD	1 mpk	4-6	336	164	94	427
XXV		20% HPβCD	1 mpk	4-6	257	126	98	430
N^2-ethyl-6-(methoxyamino)-N^4-n-propyl-1,3,5-triazine-2,4-diamine		20% HPβCD	1 mpk	4-6	208	117	95	445

TABLE 7-continued

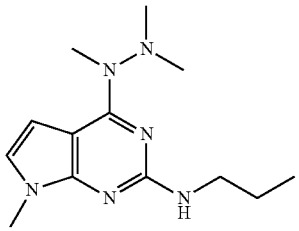
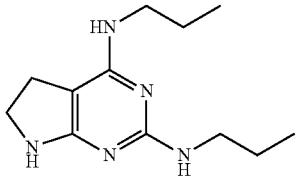
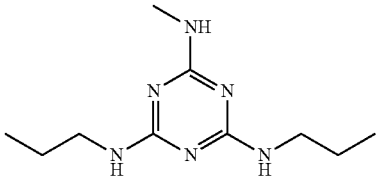
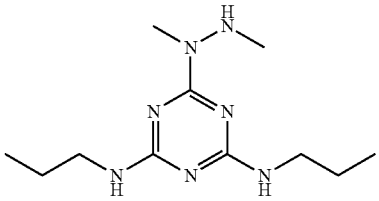
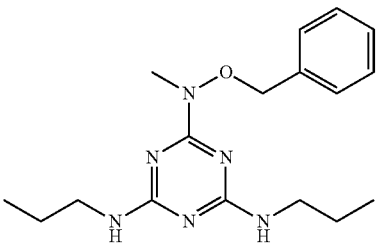
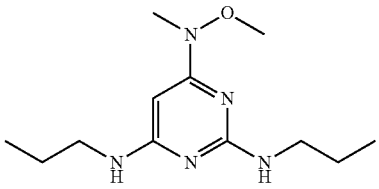
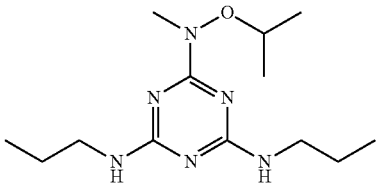
Cmpd	Structure	Formulation	Dose	pH	MV MPR	MV ΔMV	CV MAP (mm Hg)	CV Heart Rate (B/min)
7-methyl-N-n-propyl-4-(1,2,2-trimethyl-hydrazinyl)-7H-pyrrolo[2,3-d] pyrimidin-2-amine		20% HPβCD	1 mpk	4	175	83	93	372
		15/65/20	1 mpk	5	167	89	87	376
N^2,N^4-di-n-propyl-6,7-dihydro-5H-pyrrolo [2,3-d]pyrimidine-2,4-diamine		20% HPβCD	1 mpk	4	175	93	93	385
		20% HPβCD	1 mpk	6	263	167	99	381
N^2-methyl-N^4,N^6-di-n-propyl-1,3,5-triazine-2,4,6-triamine		15/65/20	1 mpk	5	145	106	92	285
6-(1,2-dimethylhydrazinyl)-N^2,N^4-di-n-propyl-1,3,5-triazine-2,4-diamine		20% HPβCD	1 mpk	5	338	216	97	329
LIII, LIV		20% HPβCD	1 mpk	4-6	288	179	96	403
6-(meth-oxy(methyl)amino)-N^2,N4-di-n-propylpyrimidine-2,4-diamine		20% HPβCD	1 mpk	4-6	355	243	90	356
6-(isopro-poxy(methyl)amino)-N^2,N^4-di-n-propyl-1,3,5-triazine-2,4-diamine		20% HPβCD	1 mpk		271	209	98	378

TABLE 7-continued

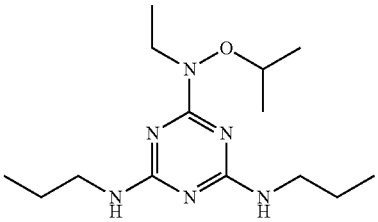
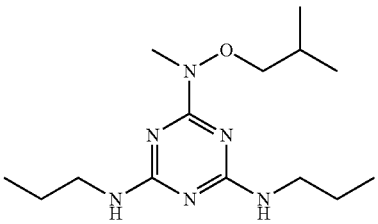
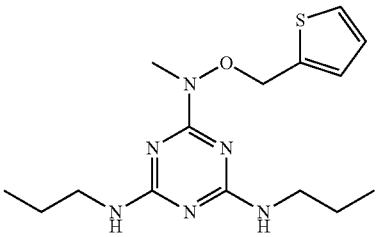
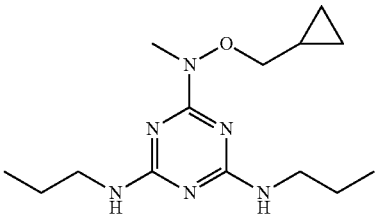
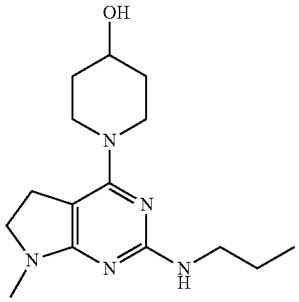
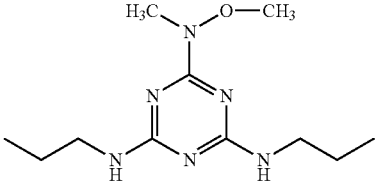
Cmpd	Structure	Formulation	Dose	pH	MV MPR	MV ΔMV	CV MAP (mm Hg)	CV Heart Rate (B/min)
6-(ethyl(isopropoxy)amino)-N^2,N^4-di-n-propyl-1,3,5-triazine-2,4-diamine		20% HPβCD	1 mpk	4	308	191	89	351
6-(isobutoxy(methyl)amino)-N^2,N^4-di-n-propyl-1,3,5-triazine-2,4-diamine		20% HPβCD	1 mpk	4	248	133	87	351
6-(methyl(thiophen-2-ylmethoxy)amino)-N^2,N^4-di-n-propyl-1,3,5-triazine-2,4-diamine		20% HPβCD	1 mpk	4	213	110	112	411
6-((cyclopropylmethoxy)(methyl)amino)-N^2,N^4-di-n-propyl-1,3,5-triazine-2,4-diamine		20% HPβCD	1 mpk	4	261	152	98	362
CLII, CLIII		20% HPβCD	1 mpk	4	49	0.33	79	245
XXXV, XXXVI		15% DMA 85% D5W	1 mpk	5-6	207	135	87	268

TABLE 7-continued

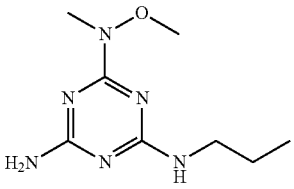
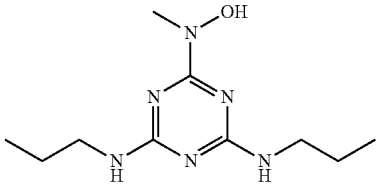
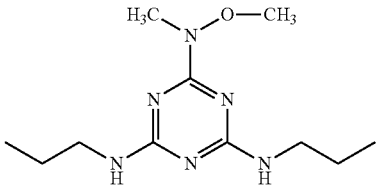
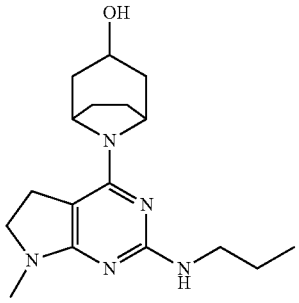
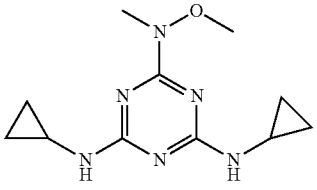
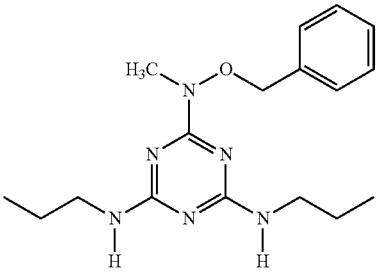
Cmpd	Structure	Formulation	Dose	pH	MV MPR	MV ΔMV	CV MAP (mm Hg)	CV Heart Rate (B/min)
XLVII		20% HPβCD	1 mpk	5	150.73	61.48	109.6	350.52
XLVIII		15/65/20	1 mpk	5	114	30.13	98	300
XXXV, XXXVI		20% HPβCD	1 mpk	4-5	344	210	90	321
CLV, CLVI		20% HPβCD	1 mpk	4	170	57	111	349
XXXIII		20% HPβCD	1 mpk	4-6	147	53	89	480
LIII, LIV		20% HPβCD	1 mpk	4-6	288	179	96	403

TABLE 8

				MV		CV MAP (mmHg)		CV HR (B/min)	
Compound	Formulation	Dose	pH	MPR	DMV	BL	DE	BL	DE
CXX, CXXI	20% HPβCD	1 mpk	4.5	355	243	107	2.3	293	17
CLXX, CLXXI	20% HPβCD	1 mpk	4	175	83	93	−1.0	372	−9.0
CXLIX, CL	20% HPβCD	1 mpk	4	175	93	93	−1.7	385	−28
CLXX, CLXXI	15/65/20	1 mpk	5	167	89	87	1.0	376	−3.8
XXXV, XXXVI	20% HPβCD	1 mpk	5	267	164	101	−1.7	335	43
CXLIX, CL	20% HPβCD	1 mpk	6	263	167	99	−3	381	−18
XXXI	20% HPβCD	1 mpk	4	140	43	101	2.6	372	2.5
CXIII, CXIV	20% HPβCD	1 mpk	4	167.1	46	101	−4	368	23
CXV, CXVI	15/65/20	1 mpk	4	155.7	33.8	97	−5	374	−5
CXVII, CXVIII	20% HPβCD	1 mpk	4.5	143.8	15	92	−8	365	−2
LXXII, LXXIII	20% HPβCD	1 mpk	4	176	82	358	8	108	−4
LXXVI, LXXVII	20% HPβCD	1 mpk	4	308	191	351	15	89	−2
LXXXII, LXXXIII	20% HPβCD	1 mpk	4	248	133	351	19	87	10
LXXXIV, LXXXV	20% HPβCD	1 mpk	4	213	110	411	15	112	−1
XCI, XCII	20% HPβCD	1 mpk	4	261	152	362	63	98	−2

Example 122

Effect of Compound (XXXVI) on Benzodiazepine-Induced Respiratory Depression (BIRD)

In one aspect, the objective of the current study was to evaluate the effects of an intravenous infusion of compound (XXXXVI) on respiratory depression, induced by midazolam in rats.

Procedures:

Compound (XXXVI) was dissolved in 20% hydroxypropyl-beta-cyclodextran in sterile water, and titrated to a pH of 4-8 using pH paper and NaOH or HCl, resulting in a clear, stable solution at a concentration of 1.5 mg/mL. Other compounds: morphine sulfate, supplied as 10 mg/mL solution by (Baxter, Inc) and midazolam, supplied as 5 mg/mL solution (Hospira, Inc.)

Materials:

Male Sprague-Dawley rats (Harlan, Inc.), 250-350 g at time of dosing, surgically prepared by Harlan with jugular vein cannulas. 12 Chamber Plethysmography System with temperature/humidity compensation (Epstein et al., 1980, J. Apply Physiol. 49:1107-1115); From Buxco, Inc. (PLY 3223; Buxco, Inc, Wilmington, N.C., USA) Biosystem XA, software, v2.11.1. Customized 12 site automated infusion system (Harvard Apparatus; Instech, Inc)

Methods:

Rat whole body plethysmography was used to evaluate and quantify minute ventilation and the pattern of breathing. A respiratory waveform was generated from the exchange of air between the animal and the chamber. This exchange induced changes in air volume that were measured with a pressure transducer, constituting the respiratory waveform. Atmospheric temperature and humidity were also measured using temperature and humidity probes which sampled chamber conditions. A compensation factor was then determined and applied to the respiratory waveform using a standardized algorithm (Epstein et al., 1980, J. Apply Physiol. 49:1107-1115) to compensate for respiratory conditioning, which was reported as the parameter COMP (see appendix).

All animals were acclimated to plethysmography chambers for at least 1 hour, or until animals were no longer restless (up to 2 hours) prior to data collection. Bolus intravenous (IV) dosing was administered at a rate of 5-10 seconds per dose, and catheters were flushed with 350 μL of sterile saline to be sure of complete drug delivery. All 12 animals (6 vehicle; 6 drug treated) were dosed simultaneously within one 60 second period. For intravenous infusions, vehicle or test compound was prepared at a 0.1 mg/mL stock as described, and administered with Harvard apparatus infusion pumps. Compound (XXXVI) was given via infusion over a 30 minute period at a rate of 20 μL/min/0.3 kg, to give a dose of 0.1 mg/kg/min, beginning 5 minutes after the bolus IV administration of midazolam. None of the included studies were performed blinded, due to the logistics of simultaneously dosing multiple animals. All plethysmography data was recorded automatically by the Buxco equipment.

Statistical Analysis:

Respiratory data were collected on a breath-by-breath basis and averaged into 1 min time bins for data analysis. For each designated acquisition phase, which is the time between doses, percent change from pre-treatment baseline values were calculated for each cohort on multiple ventilatory parameters including respiratory frequency (f), tidal volume (TV), accumulated volume (AV), minute ventilation (MV),

inspiratory time (Ti), expiratory time (Te), peak inspiratory flow (PIF), peak expiratory flow (PEF), relaxation time (RT), end inspiratory pause (EIP), end expiratory pause (EEP), delta volume (DV), expiratory flow at 50% TV (EF50), rejection index (Rinx), compensation (Comp), enhanced pause (Penh), pause (PAU), PEF rate (Rpef), relative humidity (RH), and atmospheric temperature (Temp).

Each parameter was compared to vehicle in order to calculate percent difference, using area under the curve (AUC) and peak response values for each defined acquisition period using a customized visual basic restructure analysis macro. Additionally, percent reversal of drug induced respiratory depression by compound was calculated using the mean respiratory depression derived from all vehicle (or untreated) animals, compared to the mean pre-treatment baselines of all animals in the study.

Results:

Compound (XXXVI) (0.1 mg/kg/min) reversed the BIRD induced decrease in MV by 100 by the end of the infusion period (t=38). Compound (XXXVI) had an effect to reverse the effects of midazolam on MV (FIG. **15**), TV (FIG. **16**), AV, Te, PIF, PEF, and EEP, and had no obvious effect on f (FIG. **17**), Ti, RT, EIP, dv, EF50, Rinx, Comp, Penh, PAU, Rpef, RH, and Temp. Small increases in minute ventilation in all groups were often seen during IV bolus injections which were treated as injection artifacts due to animal arousal, and had no other apparent impact on ventilation.

Potential adverse events were monitored, and there were no adverse behavioral effects observed following administration of compound (XXXVI) at the doses tested. The results of these experiments demonstrated that compound (XXXVI) reverses midazolam induced-respiratory depression and appears to be well tolerated in rats at the doses tested.

Example 123

Effect of Compound (XXXVI) on Hypercapnia

In one aspect, the objective of the study was to evaluate the effects of Compound (XXXVI), administered by intravenous infusion, on the hypercapnic ventilatory response (HCVR) in rats.

Vehicle, 20% hydroxypropyl-beta-cyclodextran in sterile water, was added to pre-weighed compound and mixed thoroughly, resulting in a clear solution. A 1.5 mg/mL stock was created to give an infusion dose of 0.10 mg/kg/min. A separate 0.45 mg/mL stock was created to give an infusion dose of 0.03 mg/kg/min. All compound solutions and vehicles were titrated to have a pH between 4-8, using a pH paper and titrating with NaOH or HCl solution.

Male Sprague-Dawley rats (Harlan, Inc.), 250-350 g at time of dosing, had been surgically prepared by Harlan to contain jugular vein cannulas. A 12 chamber plethysmography system with temperature/humidity compensation (Epstein et al., 1980, J. Apply Physiol. 49:1107-1115) from Buxco, Inc. (PLY 3223; Buxco, Inc, Wilmington, N.C., USA) using Biosystem XA software, v2.11.1 and a customized 12 site automated infusion system (Harvard Apparatus, Instech, Inc). Gas mixer (CWE Inc.) was used for this experiment to give concentrations of hypercapnia (3% CO_2, 21% O_2, balance nitrogen). Three tanks containing 100% O_2, CO_2, and nitrogen were attached to the gas mixer, with the customized gas mixture fed to each plethysmograph at a rate of 2 L/min.

Rat whole body plethysmography was used to evaluate and quantify minute ventilation and the pattern of breathing. A respiratory waveform was generated from the exchange of air between the animal and the chamber. This exchange induced changes in air volume that were measured with a pressure transducer, constituting the respiratory waveform (Lomask M., 2005, "Respiration measurement in the whole body plethysmography," Buxco Inc., retrieved from http://www dot buxco dot com/downloads/LomaskWBP dot pdf). Atmospheric temperature and humidity were also measured using temperature and humidity probes that sampled chamber conditions. A compensation factor was then determined and applied to the respiratory waveform using a standardized algorithm (Epstein et al., 1980, J. Apply Physiol. 49:1107-1115) to compensate for respiratory conditioning, which was reported as the parameter COMP.

All animals were acclimated to plethysmography chambers for at least 1 hour, or until animals were no longer restless (up to 2 hours prior to data collection). Minute ventilation (MV) was calculated by the Biosystem XA software from direct measurements of tidal volume (TV) and respiratory frequency (f) using the formula MV=TV×f. Minute ventilation (mL/min) is a common endpoint for evaluating ventilatory performance. The protocol included compound (XXXVI) given as a 20 minute infusion, followed by a 20 minute exposure to 3% hypercapnia in addition to the compound (XXXVI) infusion. After 20 minutes of hypercapnia, the compound (XXXVI) infusion continued for an additional 10 minutes, resulting in a total compound (XXXVI) infusion time of 50 minutes. Each experiment included 6 animals receiving compound (XXXVI) (0.03 or 0.10 mg/kg/min) tested against 6 animals receiving vehicle, all of which were challenged with hypercapnia (3%). Animals were continuously monitored and observed for adverse behavioral effects. Such findings were recorded into laboratory notebooks for each individual animal tested.

Statistical Analysis

Respiratory data was collected on a breath-by-breath basis and averaged into 1 min time bins for data analysis. For each designated acquisition phase, which is the time between doses, AUC percent change from pre-treatment baseline values were calculated for each cohort on multiple ventilatory parameters including, respiratory frequency (f), tidal volume (TV), accumulated volume (AV), minute ventilation (MV), inspiratory time (Ti), expiratory time (Te), peak inspiratory flow (PIF), peak expiratory flow (PEF), relaxation time (RT), end inspiratory pause (EIP), end expiratory pause (EEP), delta volume (dV), expiratory flow at 50% TV (EF50), rejection index (Rinx), compensation (Comp), enhanced pause (Penh), pause (PAU), PEF rate (Rpef), relative humidity (RH), and atmospheric temperature (Temp). Each parameter was compared to vehicle in order to calculate percent difference, using area under the curve (AUC) and peak response values for each defined acquisition period using a customized visual basic restructure analysis macro (Lopotosky, S. Galleon Buxco Restructure tool, v5.2, 2008). All of the data analysis for the single, stand-alone studies was done using the restructure analysis macro (Lopotosky, S. Galleon Buxco Restructure tool, v5.2, 2008). All merged data analysis with both doses was performed in Graphpad Prism for MV, TV, and f only, for hypercapnia studies. Additionally, percent increase was calculated based on the cohort average immediately prior to the hypercapnic challenge to the group's peak response. For merged hypercapnia studies, t=62 to t=67 was used.

Results and Discussion:

Administration of compound (XXXVI) stimulated respiration in naive animals at 0.10 mg/kg/min, with little or no effect at 0.03 mg/kg/min. Furthermore, when animals received compound (XXXVI), exposure to hypercapnia (3% CO_2) resulted in an increased MV response in a dose depen-

dent manner when compared to vehicle treatment alone. Compound (XXXVI), at 0.03 or 0.1 mg/kg/min IV, resulted in the augmentation of the hypercapnia ventilatory response (HCVR), at the higher dose of 0.10 mg/kg/min, with no effect at 0.03 mg/kg/min. The 0.03 mg/kg/min and vehicle groups resulted in a 60% facilitation of the HCVR, from t=62 to t=67. The 0.10 mg/kg/min demonstrated an increase of 44% in MV during the HCVR challenge at the same time points, in addition to an elevated MV due to compound (XXXVI) alone. Compound (XXXVI) at 0.03 mg/kg/min against HCVR resulted in an effect on PIF only, with no discernable change in f, TV, AV, MV, Ti, Te, PEF, RT, EIP, EEP, dv, EF50, Rinx, Comp, penh, PAU Rpef, RH, or Temp. The 0.10 mg/kg/min dose showed an effect on f, TV, AV, MV, Ti, Te, PIF, PEF, EIP, EF50, Comp, and RH, with no discernable change in EEP, dV, Rinx, Penh, PAU, Rpef, and Temp. These data suggest that compound (XXXVI), given as an infusion, may enhance the ventilatory effects of hypercapnia, resulting in facilitation of ventilation during acute hypoxemia. In addition, there were no adverse clinical observations associated with compound (XXXVI) at the doses tested.

Example 124

Effect of Compound (XLII) on Respiratory Rate, Tidal Volume, Minute Volume and 5-min Average Change in Minute Volume in Rat

General Methods:

Adult male Sprague Dawley rats (250-350 g body weight; Harlan, Indianapolis, Ind., USA) were anesthetized with isoflurane in oxygen. The femoral vein was cannulated to administer compounds. Body temperature was maintained between 36.0 and 37.2° C. throughout the experiment. The cervical portion of the trachea was cannulated and attached to a rat-sized pneumotachometer for measuring tracheal airflow. After surgical instrumentation was complete, isoflurane was slowly discontinued, while urethane (1.8 g/kg) was administered intravenously over 10-15 minutes to maintain anesthesia. Rats were permitted to breathe spontaneously and inspire 100% oxygen. At least 30-45 minutes after discontinuing isoflurane, baseline tracheal airflow was recorded for 20-30 minutes prior to compound administration. Vehicle and test compound were administered intravenously over 15-25 seconds and at a constant volume (1 mL/kg). At least 15-min elapsed between each dose of compound administered. Tracheal airflow was recorded continuously before, during and after compound administration. Tidal volume was derived from the integral of airflow, and respiratory rate was derived from the number of inspiratory cycles per minute. Minute volume was calculated as the product of tidal volume and respiratory rate (FIG. **32**).

Results:

Compared to vehicle, Compound (XLII) increased both respiratory rate and tidal volume, and their product minute volume.

Example 125

Effect of Compound (CLXXII) on Respiratory Rate, Tidal Volume, Minute Volume and 5 min Average Change in Minute Volume in Rat

General Methods

See Example 124 above

Results

Compared to vehicle, Compound (CLXXII) increased both respiratory rate and tidal volume, and their product minute volume.

Example 126

Effect of Compound (XXXV) on BK Potassium Channel Current in GH3 Cells

Experimental Procedures

Stock solutions of the test article were prepared in dimethylsulfoxide (DMSO) and stored frozen. Test article concentrations were prepared fresh daily by diluting stock solutions into a physiological saline solution (composition in mM): KCl, 125; $CaCl_2$, 8.5; HEPES, 10; EGTA, 11.25; pH adjusted to 7.2 with KOH (prepared weekly and refrigerated until used). All test solutions contained 0.1% DMSO.

GH3 cells were grown in culture using standard procedures. Ionic currents were measured from inside-out patches of GH3 cells. Channel activity was monitored in symmetrical K^+ conditions with a holding potential of −60 mV. Activities before and in the presence of each concentration of test articles were recorded for ≥3 minutes, respectively.

Data Analysis

Data acquisition and analyses were performed using the suite of pCLAMP (Ver. 8.2) programs (MDS-AT, Sunnyvale, Calif. Percent inhibition by test article was calculated using the following equation:

$$100*(NPo_{control}-NPo_{test})/NPo_{control}$$

Results

Compound (XXXV) was evaluated at 1 and 10 and almitrine, a positive control, was evaluated at 1 μM. Channel effects were estimated from the change in activities at steady-state after application of test articles. The effect of Compound (XXXV) is summarized in Table 9. A sample recording before and after 1 μM (XXXV) and all points histogram is showed in the FIG. **21**.

TABLE 9

Effect of Compound (XXXV) on the single BK potassium channel expressed in GH3 cells recorded in inside-out patches

		NPo				Inhibition (%)		
	Channel Amplitude		(XXXV) (mM)		Almirite (mM)	(XXXV) (mM)		Almirite (mM)
Cell ID	(pA)	control	1	10	1	1	10	1
C 05 SP 101008 0000	−14.3	0.013	0.0076	0.0057	0.0011	41.5	56.2	91.5

TABLE 9-continued

Effect of Compound (XXXV) on the single BK potassium channel expressed in GH3 cells recorded in inside-out patches

		NPo				Inhibition (%)		
	Channel Amplitude		(XXXV) (mM)		Almirite (mM)	(XXXV) (mM)		Almirite (mM)
Cell ID	(pA)	control	1	10	1	1	10	1
E 05 SP 101008 0000	−17.8	0.042	0.037	0.023	0.00090	11.9	45.2	97.9
A 05 SP 101012 0000	−15.5	0.037	0.032			13.5		
B 05 SP 101012 0000	−16.6	0.066		0.051			22.7	
Average	−16.0	0.040	0.014	0.020	0.0010	22.3	41.4	94.7
SD	1.4	0.022	0.016	0.027	0.0001	16.7	17.0	4.5

NPo: total channel open probability.

Inhibition (%) = 100 * ($NPo_{control}$ − NPo_{test})/$NPo_{control}$

Washout was performed to the patch C 05 SP 101007 0000 before and after application of 1 μM almitrine, to the patch B 05 SP 101012 000 after application of 10 μM (XXXV).

Summary and Conclusions

As illustrated in FIG. **34**, Compound (XXXV) exerted concentration—dependent inhibition of the native BK channel expressed in GH3 cells. At the highest concentration tested (10 μM) Compound (XXXV) reduced total channel open probability by 42%.

Example 127

Effect of Compounds on Thallium Flux Through Rat TASK-1 (KCNK1) and Human TASK-3 (KCNK9) Channels

Experimental Procedure

(a) Seed Cell Plates:

CHO cells expressing recombinant tetracycline-inducible rat KCNK3/TASK-1 (KCNK3-CHO cells) were plated in poly-D-lysine coated 384-well plates at a density of 6,000 cells/well (50 μl/well of 120,000 cells/mL) and allowed to incubate for 72 hrs in the presence of 5% CO_2 and 1 μg/mL tetracycline at 30° C. HEK cells expressing recombinant tetracycline-inducible human KCNK9/TASK-3 (KCNK9-HEK cells) were plated at a density of 15,000 cells per well (50 μl/well, 300,000 cells/mL) and incubated overnight at 37° C. and 5% CO_2 in the presence of 1 μg/mL tetracycline.

(b) Preparation of Compound Containing Plates:

Drug plates were generated from 10 mM 100% DMSO mother plates. Compounds were transferred to 384-well drug plates and titrated with sequential 1:3 dilutions in 100% DMSO for a total of 9 dilutions. A dilution plate is made by taking 2.4 μl from the mother plate and adding to 17.6 μA of assay buffer for a 12% DMSO dilution plate. From this dilution plate, a final daughter plate is generated by taking 1 μA of the dilution plate and adding to 40 μA of assay buffer for a final DMSO concentration of 0.3%. Each compound was evaluated in duplicate and the concentration range evaluated was 3 nM to 30 μM.

Assay Protocol:

When thallium was added to extracellular solution with a stimulus to open channels, Tl^+ flowed down its concentration gradient into the cells and channel activity was detected with an indicator dye, FluxOR™, that increased in cytosolic fluorescence. The FluxOR™ indicator dye was loaded into cells as a membrane permeable acetoxymethyl ester. On the day of each assay, FluxOR™ assay buffer was made fresh from Hank's Balanced Saline Solution (HBSS) buffered with 20 mM HEPES and pH adjusted to 7.4 with NaOH. Dye loading solution was prepared per manufacturer's instructions, and 25 μl of dye solution applied to each well with a multidropper after plating medium was removed manually from cells. Dye was allowed to load for 90 minutes at room temperature in the dark.

After the 90 min dye loading incubation and subsequent manual dye removal, 24 μl of the drug daughter plate was transfer to cell plates by a cybi-well system and then incubated at room temperature for 20 minutes to allow for equilibration within wells. Stimulation buffer was prepared from 5× chloride-free buffer, thallium, and potassium sulfate containing 5 times the desired final assay concentration of 10 mM K^+ 2.8 mM Tl^+. Cell plates were then transferred to an FDSS 6000 kinetic imaging plate reader (Hamamatsu). Fluorescence at 525 nm (excitation 488 nm) was measured for 10 seconds at 1 Hz to establish baseline for the entire plate. After baseline was established, 6 μl/well of stimulation buffer was added and fluorescence signal recorded for another 140 seconds.

Data Analysis

From the fluorescence signals in the absence and presence of Galleon compounds, Inhibitory concentration curves were plotted and IC_{50} values were estimated using a curve fitting algorithm.

Results

The results are summarized in Table 10. Using Tl^+ flux as an indicator of rat TASK-1 and human TASK-3 activity, some compounds displayed blockade of rTASK-1.

TABLE 10

IC_{50} values for inhibition of rat TASK-1 and hTASK-3

Compound	hTASK-3 IC_{50} (μM)	rTASK-1 IC_{50} (μM)
Cmpd (XXXVI)	>30	25
Cmpd (XLII)	>30	0.05
Cmpd (XLVI)	>30	0.60
Cmpd (CLXXII)	>30	0.01

TABLE 10-continued

IC_{50} values for inhibition of rat TASK-1 and hTASK-3		
Compound	hTASK-3 IC_{50} (μM)	rTASK-1 IC_{50} (μM)
Cmpd (CXXI)	>30	0.22
Cmpd (CLXX)	>30	0.16

Example 128

Effect of Compound (XXXVI) on Ventilation in Human Volunteers

Study Design:

A randomized, double-blind, placebo-controlled nine-period single-rising dose study in three alternating cohorts of healthy subjects was conducted at a single center in conformance with Good Clinical Practice. Compound (XXXVI) was synthesized according to Good Manufacturing Practice. Three (3) Cohorts of ten subjects each were randomized to treatment in a ratio of 4:1 (8 treated, 2 placebo) in a rotating panel design. Subjects in the first cohort participated in dosing periods 1, 4, and 7, with the second cohort participating in dosing periods 2, 5, and 8, and third cohort participating in dosing period 3, 6, and 9. The subjects who received placebo in their first dosing period also received placebo in their second and third dosing periods. Similarly, drug-treated subjects received drug all three dosing periods. In the first Cohort, on the first dose only, 2 subjects were dosed on Day 1 with one subject receiving active drug and one subject receiving placebo. Following a review of available safety and tolerability data (excluding PK), the remainder of Cohort 1 was dosed the following day. Subjects were dosed as detailed in Table 11 with a minimum of 1-week washout between doses.

Subjects could be screened up to 28 days before the treatment period. On the evening before dosing, subjects were admitted to the clinical site and baseline evaluations were conducted. After an overnight fast, subjects received an intravenous infusion of Compound (XXXVI) or placebo then PD, PK, and safety evaluations were conducted during the next 24 hours. Cohort 1 was enrolled to the lowest dose group and randomized to Compound (XXXVI) or placebo. Safety and tolerability evaluations for each dose included all safety assessments obtained through approximately 24 hours post-dose.

Pharmacodynamic endpoints were assessed by two independent systems consisting of Dräger Infinity patient monitoring system and a portable NOX-T3 respiratory monitoring system. The Dräger system is an inpatient intensive care/postoperative monitoring system used to monitor minute ventilation, respiratory rate (RR), tidal volume (V_T) via a pneumotachometer (Capnostat sensor), transcutaneous oxygen hemoglobin saturation (SpO_2), and end tidal carbon dioxide ($ETCO_2$) (Capnostat sensor). NOX/T3 is a portable monitoring device used for home diagnostic sleep apnea assessments. In this study, NOX device monitored RR, V_T via respiratory inductance plethysmography (RIP), SpO_2 (Nonin), actigraphy, nasal air flow, and audio recording of the dosing session. In addition to monitoring pharmacodynamic effects, the Dräger system was also used to monitor subject real-time safety. Standard safety assessments were measured repeatedly throughout the dosing session, and consisted of vital signs (BP, HR, RR, temperature (oral)), Ramsay Sedation Scale (RSS), and pulmonary function testing.

Test Product, Dose, Mode of Administration, Batch and Lot No(s): Compound (XXXVI) was prepared as a sterile product for injection ready for dilution in normal saline and administration by infusion. Doses were based on the subject's weight on a milligram per kilogram basis and were administered over a 1-, 2-, 3- or 4-hour infusion as described in Table 11. Compound (XXXVI) (Batch No. 04110511) was provided by the Sponsor as a 50 mL fill of 10 mg/mL solution (GAL-021 freebase) in 20 mM citrate for IV Injection; Saline was sourced by the Investigator from Baxter, Lot 11F03E7L.

TABLE 11

Compound (XXXVI) Doses (Infusion Time)								
	Cohort 1			Cohort 2			Cohort 3	
Period 1	1*	0.0017 mg/kg/min (1 Hr)	2*	0.005 mg/kg/min (1 Hr)	3*	0.005 mg/kg/min (1 Hr)		
Period 2	4*	0.01 mg/kg/min (1 Hr)	5*	0.016 mg/kg/min (1 Hr)	6*	0.012 mg/kg/min (2 Hr)		
Period 3	7*	0.012 mg/kg/min (3 Hr)	8*	0.009 mg/kg/min (4 Hr)	9*	0.012 mg/kg/min (1 Hr)/ 0.006 mg/kg/min (3 Hr)		

*Dose group (sequence of dose progression thoroughout study)

Reference Therapy, Dose, Mode of Administration:

Matching placebo consisted of sterile normal saline for infusion purchased by the investigator. Compound (XXXVI) formed a colorless solution and the identity was equal to sterile saline when mixed for infusion. Normal saline was infused at the same rate as treated subjects.

Duration of Treatment:

Thirty (30) subjects received single IV infusions over 1, 2, 3, or 4 hours on the first day of each of 3 periods for a total of 3 dosing days per subject. Per protocol, there was to be a minimum one week washout between doses of Compound (XXXVI) for all subjects. Actual washout periods ranged from 9 to 20 days overall.

Pharmacodynamics:

Pharmacodynamic (PD) evaluations included tidal volume, respiratory rate, minute ventilation, end-tidal CO_2, and transcutaneous hemoglobin oxygen saturation (SpO_2). Respiratory assessments were performed using both a pneumotachometer embedded in an occlusion facemask and inductance plethysmography (NOX-T3). Before the study the Dräger pneumotachometer was designated as the primary device for assessing PD respiratory results. The NOX-T3 device was included as an exploratory device for comparison to the Dräger instrument (data not presented).

At all doses, change in respiratory rate was small and usually <10%. Tidal volume changes were also small with

only the 0.016 mg/kg dose having a consistent increase <10%. PD data was collected for ~1-hr after completing the infusion. To further analyze the responses, data was pooled across treatment periods with similar infusion rates during the first hour. Dose groups 4 & 8 and 6, 7 & 9 (see Table 11) were pooled for analysis during the first 2 hours after infusion start, which was the common data acquisition period across all treatment groups. Baseline respiratory data was acquired for 1 hour before dosing and the parameters average over that period after removal of recorded regions contain artifacts by treatment-blinded polysomnographic experts before analysis. The time weighted percent change from baseline was calculated for all respiratory parameters. To further evaluate the respiratory changes, the responses were integrated over the first 2 hours after the start of the infusion. Minute ventilation did not in change significantly except at the highest infusion rate with a ~27% increase noted (FIG. **35**).

Conclusions:

Compound (XXXVI) for IV injection was generally safe and well tolerated in healthy volunteers over the dose range administered in this study (0.102-2.16 mg/kg) with durations of 1 to 4 hours). Compound (XXXVI) stimulated minute ventilation at the highest infusion rate (0.016 mg/kg/min×1 hour). Compound (XXXVI) was well-behaved pharmacokinetically following intravenous infusion in the dose range of 0.102 to 2.16 mg/kg with durations of 1 to 4 hours. Compound (XXXVI) was dose proportional for concentration at 1 hour and AUC in the dose range of 0.102 to 2.16 mg/kg with durations of 1 to 4 hours.

Example 129

Development of Liquid Formulation for (XXXVI)

A. Solubility of (XXXV) as a function of pH

Methodology

A solution of (XXXVI) of concentration ~10^7 mg/mL in 100 mM citric acid at pH 2.5 was prepared. About 150 μL aliquots of this solution were transferred into 6 separate glass HPLC vials. Each aliquot was titrated with 6N sodium hydroxide to different pH values in the range 3-5.5 (Table 12A). All the samples, including the solution at pH 2.5, were shaken overnight at room temperature using a gyratory shaker. After shaking, the samples were evaluated visually for the presence of precipitate and were then spin filtered through 0.45 μm syringe tip filters (4 mm diameter) at 3,000 rpm for 20 min. The final pH values of the filtrates were measured. The filtrates were then diluted appropriately and analyzed for (XXXV) concentration using the HPLC assay.

Results

The pH values of the samples prior to and after shaking overnight, appearance as well as (XXXV) concentrations of samples are illustrated in Table 12A. Solutions prepared at target pH values of 2.5, 3.0 and 3.5 were clear (no excess solid present) and hence the (XXXV) concentration values for these samples do not represent saturation solubility values. The pH solubility profile is shown in FIG. **19**B. The closed diamonds are saturation solubility values and open diamonds are measured concentrations in non-saturated samples (no excess solid present). The dotted line represents the best fit curve, which was plotted using Equation 1. This equation is a simplified representation of solubility equilibrium for weak bases that assumes that total solubility is much larger than the solubility of the free base in the pH range of interest:

$$\log S = \log S_0 + \log(1+10^{pKa-pH}) \qquad \text{Equation 1}$$

where, 'S' is total solubility; 'S_0' is the solubility of the free base.

Using the Microsoft Excel Solver function and a pKa value of 5.6 for (XXXVI), Equation 1 was fit to the experimentally determined pH-solubility profile. The solubility value of free base thus calculated was 0.18 mg/mL. Based on this equation, solubility (expressed as the free base) is predicted to be 10 mg/mL at about pH 3.86, 25 mg/mL at about pH 3.46, and 50 mg/mL at about pH 3.16.

TABLE 12A

pH Solubility Results

target pH	initial pH	final pH after Shaking and Filtration	appearance afterwards Shaking	(XXXVI) concentration
2.5	2.51	2.42	Clear solution	104.1
3.0	3.05	3.00	Clear solution	99.8
3.5	3.51	3.51	Clear solution	101.6
4.0	4.00	3.79	Precipitate	20.8
4.5	4.55	4.37	Precipitate	3.46
5.0	4.93	4.52	Precipitate	2.73
5.5	5.63	5.02	Precipitate	0.99

B. Stability of (XXXV) as a Function of pH

Methodology

Buffer solutions containing ca. 10 mM each of citric acid and glycine in the pH range 2-6 were prepared by titrating 250 mL of a single stock containing both buffer agents with 1N NaOH/1N HCl and removing 50 mL aliquots at predetermined pH values. XXXVI solutions at 1 mg/mL were prepared in buffers of pH 2-5 by dissolving ~25 mg of XXXVI in ~23 mL of a given buffer, adjusting the pH to the target and making up the volume to 25.0 mL in a volumetric flask. (XXXVI) solution at pH 6 was prepared similarly, but at a lower drug concentration of 0.25 mg/mL using 6.68 mg of (XXXVI).

Each of the buffered XXXV solutions was filtered through a 0.2 μm polyether sulfone syringe filter into a volumetric flask, which had been previously rinsed with 70% ethanol and dried in a laminar flow hood. Following filtration, each buffered (XXXV) solution was split into 10 aliquots (9 aliquots of 2.5 mL each and 1 aliquot of 2 mL) in 5 mL serum vials previously rinsed with 70% ethanol and dried in a laminar flow hood. Filtration and preparation of aliquots were performed in a laminar flow hood. The 2 mL aliquot of each buffered (XXXV) solution was submitted for T0 analysis (HPLC assay and related substances).

Of the nine 2.5 mL aliquots, three aliquots were stored at each 40° C. and 60° C., two aliquots were stored at 25° C. and one aliquot was stored at −70° C. Samples stored at 40° C. and at 60° C. were evaluated for physical appearance, pH, assay and related substances after 1 week, 2 weeks and 4 weeks of storage. Samples at 25° C. and −70° C. served as controls to be analyzed if necessary.

Results

The pH stability data are shown in Table 12B. All samples remained clear, colorless and particle free for up to 4 weeks of storage at both 40° C. and at 60° C. The pH values for all the samples remained relatively constant for up to 4 weeks at both storage temperatures. Both the assay and impurities data indicate that (XXXV) is significantly less stable at pH 2 as compared to the remaining pH values evaluated. No specific trends were observed in the pH range 3-6. The pH stability profiles at 60° C. are shown in FIG. **20**A (% impurities).

TABLE 12B

pH Stability of (XXXV) (GAL 021) at pH 2-5

Test	Target pH	initial	40° C. Storage 1 wk	2 wk	4 wk	60° C. Storage 1 wk	2 wk	4 wk
pH	2	1.96	2.02	1.98	2.00	2.00	1.99	2.01
	3	3.02	3.05	3.05	3.06	3.07	3.03	3.07
	4	4.00	3.94	3.96	3.96	3.94	3.96	3.97
	5	5.03	4.90	4.95	4.97	4.92	4.93	4.98
	6	6.05	5.88	6.02	5.98	5.87	6.03	6.01
Assay	2	1.02	0.997	0.975	0.970	0.929	0.754	0.754
	3	0.984	0.984	0.987	0.991	0.971	0.952	0.952
	4	1.01	0.999	1.006	1.012	0.992	0.979	0.979
	5	1.01	0.989	0.992	0.977	0.977	0.947	0.947
	6	0.260	0.257	0.253	0.251	0.247	0.234	0.234
Impurities	2	n.d.	0.58	1.48	2.49	3.83	9.89	14.34
	3	n.d.	n.d.	0.19	0.41	0.71	1.20	2.19
	4	n.d.	n.d.	0.17	0.22	n.d.	0.52	1.02
	5	n.d.	n.d.	0.26	n.d	n.d.	0.62	1.10
	6	n.d.	n.d.	0.25	0.28	0.56	0.87	1.65

n.d. = not detected

C. Stability of (XXXV) Across Narrow pH (pH 2-3.6)

Methodology

Buffer solutions containing ca. 10 mM each of citric acid and glycine in the pH range 2-3.6 were prepared by titrating 250 mL of a single stock containing both buffer agents with 1N NaOH/1N HCl and removing 50 mL aliquots at predetermined pH values. (XXXVI) solutions at 1 mg/mL were prepared in the buffers by dissolving ~25 mg of (XXXVI) in ca. 23 mL of a given buffer, adjusting the pH to the target and making up the volume to 25.0 mL in a volumetric flask. Each of the buffered (XXXV) solutions was filtered through a 0.2 μm polyether sulfone syringe filter into a volumetric flask previously rinsed with 70% ethanol and dried in a laminar flow hood. Following filtration, each buffered (XXXV) solution was split into 9 aliquots in 5 mL serum vials previously rinsed with 70% ethanol and dried in a laminar flow hood. Filtration and preparation of aliquots were performed in a laminar flow hood. Of the nine aliquots, three aliquots were stored at each 40° C. and 60° C., two aliquots were stored at 25° C. and one aliquot was submitted for T0 testing. Samples were evaluated for pH, assay and related substances at T0 and, following 1 week, 2 weeks and 4 weeks of storage at 40° C. and 60° C. Samples stored at 25° C. served as controls to be analyzed if necessary

Results

Data are summarized in Table 12C and in FIG. **20**B. The most notable result from this study is that during both 40° C. and 60° C. storage, impurity levels decreased significantly with increasing pH across the full pH range studied. This means that any increase in the pH of the formulation led to an improvement in stability within the pH range in which an aqueous formulation is feasible.

TABLE 12C

pH Stability of (XXXV) at pH 2-3.6

Test	Target	initial	40° C. Storage 1 wk	2 wk	4 wk	60° C. Storage 1 wk	2 wk	4 wk
pH	2.0	2.00	2.00	1.98	1.98	2.00	1.97	1.89
	2.3	2.33	2.36	2.32	2.33	2.36	2.34	2.28
	2.7	2.72	2.73	2.72	2.73	2.75	2.74	2.67
	3.0	3.02	3.04	3.05	3.04	3.05	3.04	3.00
	3.3	3.31	3.35	3.35	3.34	3.36	3.37	3.34
	3.6	3.62	3.64	3.70	3.64	3.65	3.70	3.64

TABLE 12C-continued

pH Stability of (XXXV) at pH 2-3.6

Test	Target	initial	40° C. Storage 1 wk	2 wk	4 wk	60° C. Storage 1 wk	2 wk	4 wk
Assay	2.0	0.981	0.959	0.955	0.956	0.913	0.851	0.768
	2.3	0.987	0.984	0.993	0.995	0.961	0.932	0.916
	2.7	0.987	0.981	0.985	1.019	0.978	1.144	0.987
	3.0	1.005	1.01	0.987	1.027	0.991	0.975	0.987
	3.3	1.019	1.02	1.01	1.047	0.997	0.995	1.027
	3.6	0.996	0.991	0.985	0.991	0.979	0.958	0.979
Impurities	2.0	0.14	0.99	1.55	2.84	3.87	7.56	13.99
	2.3	0.09	0.47	0.63	1.70	1.81	3.53	6.30
	2.7	n.d.	0.22	0.37	0.61	0.97	1.76	3.09
	3.0	n.d.	0.21	0.34	0.58	0.61	1.27	1.82
	3.3	n.d.	n.d	n.d	0.25	0.41	0.69	1.26
	3.6	0.12	n.d	n.d	0.17	0.36	0.62	0.99

n.d. = not detected

D. Evaluation of the Solubility of (XXXV) in Solvents and IV Solutions

Methodology

The solubility of (XXXXVI) and XXXV was evaluated in various solvents and IV solutions. The solvents and target concentration for solubility evaluation are shown in Table 12D. Due to limits on the API supply at the time the study was conducted, most of the solvent systems were evaluated to a level of only 100 mg/mL even if the solution was not saturated at this level. Only in water was an attempt made to reach saturation solubility, regardless of how high that solubility might be. Solubility of the salt/free base in each solvent was evaluated in duplicate.

Evaluation of solubility of (XXXVI) in 0.9% NaCl, 5% dextrose, ethanol, 30% PEG 400, 70% PEG 400 and 100% PEG 400 was carried out at a target of 200 mg/mL by adding ~100 mg of drug to 500 μL of solvent. The rest of the solubility evaluations, except water, were carried out by adding ~50 mg of drug (XXXVI or XXXV) to 500 μL of solvent. For solubility in water, drug was added with intermittent shaking to ensure that excess undissolved drug is present indicating saturation 13 mm PTFE 0.2 μm syringe filters. The filtrates were diluted appropriately and analyzed for (XXXV) concentration by HPLC.

Results

The solubility results are shown in Table 12D.

(XXXVI):

The solubility of (XXXVI) was greater than 400 mg/mLa in water and at least 200 mg/mL in IV solutions (0.9% saline and 5% dextrose). During the evaluation of solubility of the (XXXV) salt in water it was noted that ~350-400 mg the API dissolved completely in 500 μL of water; however, the sample formed a cloudy gel following ca. 2 h of shaking at room temperature that dissolved upon addition of water (~80-250 μL). Further shaking of this clear solution overnight at room temperature resulted in a cloudy gel formation. Addition of another small aliquot of water (~60-220 μL) resulted in a fluid suspension that could be filtered and analyzed for (XXXV) concentration. The exact reason for this behavior of the (XXXVI) in water was not clear but may suggest formation of a supersaturated solution. The salt was also freely soluble in ethanol and methanol and had a saturation solubility of about 68 mg/mL in acetonitrile. The solubility in 30%, 70% and 100% propylene glycol was at least 90 mg/mL. The solubility in 100% PEG-400 reached saturation at ca. 36 mg/mL but (XXXVI) was soluble to at least 90 mg/mL in 70% PEG-400 and ~198 mg/mL in 30% PEG-400.

(XXXV):

The saturation solubility of (XXV) in water was ca. 0.21 mg/mL, which is in agreement with the predicted value from the pH-solubility curve detailed in Example 129A. The solubility in ethanol, methanol and acetonitrile were ~92 mg/mL, ~102 mg/mL and ~45 mg/mL, respectively. The solubility of the free base in 5% Tween 80 dispersed system was ca. 2.7 mg/mL. The solubility in PEG-400 and propylene glycol systems appears to increase with increasing levels of the cosolvent. The free base was more soluble in propylene glycol (~90 mg/mL) than in PEG-400 (~73 mg/mL). The data suggest that the solubility of XXXV free base in propylene glycol systems would be greater than in PEG-400 systems at similar pH values.

TABLE 12D

Solubility of (XXXV) in Solvents and IV Solutions

		(XXXV) hydrogen sulfate salt			(XXXV) free base		
Solvent System	Replicate	pH	Solubility (mg/mL)	Saturation Solubility	pH	Solubility (mg/mL)	Saturation Solubility
Water	1	0.40	414.5	Y	8.80	0.206	Y
	2	0.31	441.5	Y	8.67	0.206	Y
0.9% NaCl	1	0.61	>206.2	N		Not determined	
	2	0.62	>201.4	N			
5% Dextrose	1	0.61	>201.2	N		Not determined	
	2		sample not recoverable				
Ethanol	1	NA	>211.4	N	NA	96.04	Y
	2	NA	>212.2	N	NA	87.24	Y
30% PEG-400	1	0.84	>197.0	N	4.30	17.03	Y
	2	0.82	>198.4	N	4.35	17.30	Y
70% PEG-400	1	1.68	>85.28	N	5.98	14.78	Y
	2	1.65	>91.43	N	6.09	11.76	Y
PEG-400	1	NA	35.74	Y	NA	68.3	Y
	2		sample not recoverable		NA	77.8	Y
30% Propylene	1	1.06	>93.21	N	7.36	0.87	Y
Glycol	2	1.07	>89.47	N	7.51	0.84	Y
70% Propylene	1	1.22	>87.80	N	7.93	12.4	Y
Glycol	2	1.18	>92.43	N	8.25	12.1	Y
Propylene	1	NA	>92.09	N	NA	87.0	Y
Glycol	2	NA	>94.65	N	NA	93.2	Y
Methanol	1	NA	>108.9	N	NA	94.82	Y
	2	NA	>98.52	N	NA	108.38	Y
Acetonitrile	1	NA	68.5	Y	NA	49.4	Y
	2	NA	67.1	Y	NA	39.9	Y
5% Tween 80	1				7.54	2.69	Y
	2		Not determined		7.61	2.68	Y

* NA = not applicable. Samples listed as not recoverable were lost due to filtration failures.

E. Stability of (XXXV) as a Function of Buffer Strength

Methodology

A 10 mg/mL (XXXVI) solution in 100 mM citrate buffer at pH 3.0 and a 500 mM citrate buffer at pH 3.0 were prepared. Using these two stock solutions, (XXXVI) solutions at 1 mg/mL were prepared in citrate buffers of various concentrations in the range 10-250 mM, as shown in Table 12E.

TABLE 12E

Solutions for Evaluation of Stability as a Function of Buffer Strength

Solution	Volume of 10 mg/mL (XXXVI) in 100 mM citrate pH 3.0 (mL)	Volume of 500 mM Citrate pH 3.0 (mL)	Volume of Water (mL)
1 mg/mL (XXXV) in 10 mM Citrate	2	0	QS to 20.0 mL
1 mg/mL(XXXV) in 20 mM Citrate	2	0.4	QS to 20.0 mL
1 mg/mL (XXXV) in 50 mM Citrate	2	1.6	QS to 20.0 mL

TABLE 12E-continued

Solutions for Evaluation of Stability as a Function of Buffer Strength

Solution	Volume of 10 mg/mL (XXXVI) in 100 mM citrate pH 3.0 (mL)	Volume of 500 mM Citrate pH 3.0 (mL)	Volume of Water (mL)
1 mg/mL (XXXV) in 100 mM Citrate	2	3.6	QS to 20.0 mL
1 mg/mL (XXXV) in 250 mM Citrate	2	9.6	QS to 20.0 mL

Each of the above solutions was stored in 5 mL serum vials as ca. 2.5-3 mL aliquots (total of 7 aliquots). Two aliquots were stored at each of 40° C., 60° C. and 25° C. Aliquots stored at 25° C. served as controls to be evaluated if necessary. At T0 and after 2 weeks of storage at 40° C. and at 60° C., samples were evaluated for pH, assay and related substances.

Results

Data from the buffer effect study are summarized in Table 12F. Impurity levels increased noticeably at the highest buffer concentration, but there was no effect of buffer strength at 40° C. within the range of buffer concentration relevant to the formulation.

TABLE 12F

Effect of Buffer Strength on (XXXV) Stability

Test	Buffer Strength	initial	40° C. Storage 1 wk	40° C. Storage 2 wk	60° C. Storage 1 wk	60° C. Storage 2 wk
pH	10 mM	3.16	3.26	3.21	3.3	3.2
	20 mM	3.27	3.33	3.30	3.3	3.3
	50 mM	3.28	3.34	3.29	3.3	3.3
	100 mM	3.30	3.32	3.29	3.3	3.2
	250 mM	3.21	3.25	3.22	3.2	3.2
Assay	10 mM	0.971	0.953	0.947	0.953	0.956
	20 mM	1.01	0.965	0.966	0.943	0.947
	50 mM	0.976	0.959	0.949	0.936	0.943
	100 mM	0.962	0.964	0.956	0.962	0.946
	250 mM	0.947	0.972	0.945	0.955	0.936
Impurities	10 mM	n.d.	n.d.	n.d.	0.48	0.89
	20 mM	n.d.	0.13	n.d.	0.58	0.88
	50 mM	n.d.	n.d.	n.d.	0.59	0.87
	100 mM	n.d.	0.26	0.38	0.73	1.20
	250 mM	n.d.	0.21	0.56	1.03	1.80

n.d. = not detected

F. Prototype Formulations

Methodology

A total of nine (XXXV) prototypes were prepared that included a simple buffered solution, co-solvent systems as well as non-aqueous solutions. These are shown in Table 12G. Prototypes 6, 7 and 8 resulted in significant precipitation during preparation and hence were not evaluated further. The remaining prototypes were stored at 2-8° C., room temperature, 40° C. and 60° C. in serum vials. Samples stored at 2-8° C. and room temperature were analyzed at selected time-points.

Results

Data gathered to date are provided in Table 12G.1-12G.6. The aqueous buffered formulation (Prototype #1) has been found to be stable at room temperature and under refrigeration, but some degradation has been observed under accelerated conditions. Of special concern is that a very small amount of precipitate, described as one or two snowflake-like particles, has been observed after 2 weeks at 60° C. and after 8 weeks at 40° C. Since the appearance of this precipitate during 60° C. storage coincided with an actual decrease in the primary impurity peak, the possibility of the degradant being insoluble is under investigation.

(XXXV) was unstable in Prototype #2, 50% PEG/50% water without pH adjustment. The instability was presumably due to the low pH of this formulation. The drug was found to be much more stable in Prototype #3, which has the same 50% PEG base but also contains sufficient sodium hydroxide to bring the apparent pH up to about 4.5. Similarly, good stability was seen in Prototype #9, 70% propylene glycol/30% water with ammonium acetate buffer to bring the apparent pH to about 5.3. Good stability was also seen in simple solutions of the salt in PEG or propylene glycol, presumably due to the absence of water as a reactant.

TABLE 12G

(XXXVI) Prototypes for Stability Evaluation

Prototype #	Description
1	35 mg/mL (XXXV)•H_2SO_4 in 50 mM citrate, pH 3.0
2	35 mg/mL (XXXV)•H_2SO_4 in 50% v/v PEG-400 in water
3	35 mg/mL (XXXV)•H_2SO_4 in 50% v/v PEG-400 in water adjusted to pH 4.4 with 1N NaOH
4	30 mg/mL (XXXV)•H_2SO_4 in 100% PEG-400
5	35 mg/mL (XXXV)•H_2SO_4 in 100% propylene glycol
6	35 mg/mL (XXXV)•H_2SO_4 in 50% v/v PEG-400 in acetate solution (~40 mM sodium acetate and ~100 mM sodium hydroxide)
7	35 mg/mL (XXXV)•H_2SO_4 in 50% v/v propylene glycol in acetate solution (~40 mM sodium acetate and ~100 mM sodium hydroxide)
8	35 mg/mL (XXXV)•H_2SO_4 in 50% v/v PEG-400 in acetate solution (~40 mM ammonium acetate and ~100 mM ammonium hydroxide)
9	35 mg/mL (XXXV)•H_2SO_4 in 70% v/v propylene glycol in acetate solution (~40 mM ammonium acetate and ~100 mM ammonium hydroxide)

TABLE 12G.1

Stability of (XXXV) Formulation Prototype #1
Formulation #1:
35 mg/mL (XXXV)•H_2SO_4 in 50 mM citrate, pH 3.0

Parameter	Temperature	Initial	1 week	2 weeks	4 weeks
pH	2-8° C.	2.97	3.04	—	—
	RT		3.04	—	3.07
	40° C.		2.99	3.01	3.06
	60° C.		3.01	3.02	2.97
Assay	2-8° C.	35.73	36.22	NA	NA
	RT		35.93	—	35.31
	40° C.		34.83	35.40	33.96
	60° C.		37.90	34.63	31.89
Impurities	2-8° C.	n.d.	n.d.	—	—
	RT		n.d.	—	n.d.
	40° C.		n.d.	0.35	0.52
	60° C.		0.73	0.49	0.51

TABLE 12G.2

Stability of (XXXV) Formulation Prototype #2
Formulation #2:
35 mg/mL (XXXV)•H_2SO_4 in 50% v/v PEG-400 in water

Parameter	Temperature	Initial	1 week	2 weeks	4 weeks
pH	RT	2.00	2.01	NA	2.07
	40° C.		2.04	2.03	2.11
	60° C.		2.04	2.14	2.36
Assay	RT	33.93	35.26	NA	34.47
	40° C.		35.30	34.81	33.92
	60° C.		33.70	31.77	27.45
Impurities	RT	n.d.	0.39	0.39	1.10
	40° C.		0.92	0.92	2.53
	60° C.		2.11	2.11	11.99

TABLE 12G.3

Stability of (XXXV) Formulation Prototype #3
Formulation #3:
35 mg/mL (XXXV•H_2SO_4 in 50% v/v PEG-400 in water adjusted to pH 4.4 with 1N

Parameter	Temperature	Initial	1 week	2 weeks	4 weeks
pH	2-8° C.	4.38	4.51	NA	NA
	R		4.40	NA	4.52
	40°		4.20	4.43	4.41
	60°		4.16-3.88	4.22	4.27
Assay	2-8° C.	22.17	37.31	NA	NA
	R		36.23	NA	36.03
	40°		35.66	36.53	35.96
	60°		35.33	34.70	34.14
Impurities	2-8° C.	n.d.	n.d.	NA	NA
	R		n.d.	NA	n.d.
	40°		n.d.	n.d.	0.90
	60°		0.62	1.50	1.91

TABLE 12G.4

Stability of (XXXV) Formulation Prototype #4
Formulation #4:
30 mg/mL (XXXV)•H_2SO_4 in 100% PEG-400

Parameter	Temperature	Initial	1 week	2 weeks	4 weeks
Assay	2-8° C.	27.94	30.18	NA	NA
	RT		30.20	NA	30.31
	40° C.		29.91	29.89	30.19
	60° C.		29.24	29.60	29.97
Impurities	2-8° C.	n.d.	n.d.	NA	NA
	RT		n.d.	NA	n.d.
	40° C.		n.d.	n.d.	n.d.
	60° C.		0.50	0.50	0.37

TABLE 12G.5

Stability of (XXXV) Formulation Prototype #5
Formulation #5:
35 mg/mL (XXXV)•H_2SO_4 in 100% propylene glycol

Parameter	Temperature	Initial	1 week	2 weeks	4 weeks
Assay	2-8° C.	28.97	36.22	NA	NA
	RT		36.23	NA	36.00
	40° C.		35.16	35.63	36.03
	60° C.		35.56	35.33	35.56

TABLE 12G.5-continued

Stability of (XXXV) Formulation Prototype #5
Formulation #5:
35 mg/mL (XXXV)•H_2SO_4 in 100% propylene glycol

Parameter	Temperature	Initial	1 week	2 weeks	4 weeks
Impurities	2-8° C.	n.d.	n.d.	NA	NA
	RT		n.d.	NA	n.d.
	40° C.		n.d.	0.12	0.27
	60° C.		0.23	0.74	0.99

TABLE 12G.6

Stability of (XXXV) Formulation Prototype #9
Formulation #9:
35 mg/mL (XXXV)•H_2SO_4 in 70% v/v propylene glycol in acetate solution (~40 mM)

Parameter	Temperature	Initial	1 week	2 weeks	4 weeks
pH	2-8° C.	5.28	5.27	NA	
	RT		NA	NA	
	40° C.		5.26	5.35	
	60° C.		5.25	5.34	
Assay	2-8° C.	35.50	34.40	NA	
	RT		NA	NA	
	40° C.		34.36	34.62	
	60° C.		34.69	34.55	
Impurities	2-8° C.	n.d.	n.d.	NA	
	RT		NA	NA	
	40° C.		n.d.	n.d.	
	60° C.		n.d.	0.39	

G. Final Formulation and GMP Drug Product Stability Data

The final formulation is shown in Table 12H. Stability data for drug product formulation in accord with Table 12H are shown in Table 12I.1 and 12I.2 at 25° C./60% RH and 2-8° C., respectively. The levels of N-(4,6-Bis(propylamino)-1,3,5-triazin-2-ol (CLXXVIII) measured during the stability study are shown in Table 12J.

TABLE 12H

Final Formulation for (XXXV)

Component (XXXV)	Function Active	Concentration 10 mg/mL (as free base)	% w/w 1.386 (as salt)
Citric acid, Anhydrous, USP	Buffer	20 mM	0.384
1N Sodium Hyrdoxide	adjust pH to 3.2	q.s.	q.s.
Sterile Water for Injection, USP	solvent	q.s.	q.s.

TABLE 12I.1

Long-term stability results (25° C./60% RH) for Formulated (XXXVI), GMP lot# 041I0511[a]

		Months					
Test	Specification	0	1 (I)*	3 (I)*	6 (I)*	6 (U)**	9 (I)*
Appearance of Solution	[b]	(3)[b]	(2)[b]	(2)[b]	(2)[b]	(2)[b]	(2)[b]
pH	2.7-3.6	(3) 3.1, 3.1, 3.1	(2) 3.1, 3.1	(2) 3.1, 3.1	(2) 3.1, 3.2	(2) 3.1, 3.2	3.1, 3.1
Osmolality	Report Results, mOsm/kg	(3) 126, 126, 127	(2) 125, 125	2) 128, 128	2) 127, 127	2) 127, 128	125, 125

TABLE 12I.1-continued

Long-term stability results (25° C./60% RH) for Formulated (XXXVI), GMP lot# 041I0511[a]

Test	Specification	Months 0	1 (I)*	3 (I)*	6 (I)*	6 (U)**	9 (I)*
Vial Appearance	[d]	(2)[e]	(2)[e]	(2)[e]	(2)[e]	(2)[e]	(2)[e]
Identification by Retention Time	Retention time between 0.98 and 1.02 relative to standard	(3) 0.99. 0.99, 0.99	(2) 1.00 1.00	(2) 1.00, 1.00	(2) 1.00, 1.00	(2) 1.00, 1.00	1.00 1.00
Assay	90-110% of label claim	(3) 99.1, 99.1, 99.3	(2) 97.9%, 97.9%	(2) 97.5%, 98.1%	(2) 99.3% 97.4%	(2) 98.2% 98.3	(2) 96.2%, 96.2%
Related Substances	NMT 1.00% for any single related substance NMT 3.00% total related substances	(3) <0.10% <010% <0.10% Totals <0.10 all	(2) RRT 0.672: 0.15% RRT 0.672: 0.15% Total: 0.15 Total: 0.15	2) RRT 0.672: 0.44% RRT 0.672: 0.44% Total: 0.44 Total: 0.44	2) RRT 0.672: 0.82% RRT 0.672: 0.82% Total: 0.82 Total: 0.82	2) RRT 0.672: 0.81% RRT 0.672: 0.81% Total: 0.81% Total: 0.81%	RRT 0.672: 1.16 RRT 0.672: 1.16 Total: 1.16 Total: 1.16
Subvisible Particulates	[f]	(3) 207, 37 122, 7 177, 0		(3) 418, 0 306, 0 282, 0	(3) 78, 0 99, 0 54, 0		
Weight Loss (5 vials)	NMT 2.5 g loss in weight		(5) 0.0 all	(5) 0.0 all			(5) 0.0 all

[a]Package components for GMP batch: 50 mL clear, 20 mm opening, USP Type 1 glass (Wheaton 223745); Gray butyl teflon 2 coated stopped. 4416/50 (West 1014 4937); flip off seal, 20 mm, 3766 white, 8-bridge (West 5420 3028).
[b]Colorless to pale yellow liquid. Clear and particle free
[c]Colorless liquid. Clear and particle free
[d]Vial free of cracks, stopper securely in place and no sign of leakage
[e]Both vials free from crack stoppers securely in place, no sign of leakage
[f]For each container: NMT 6000 ≥ 10 μm particles; NMT 600 ≥ 25 μm
*(I) = inverted;
**(U) = upright

TABLE 12I.2

Long-term stability results (5° C./Ambient) for Formulated (XXXVI), GMP lot# 041I0511[a]

Test	Specification	Months 0	1 (I)*	3 (I)*	6 (I)*	6 (U)**	9 (I)*	10
Appearance of Solution	[b]	(2)[b]	(2)[b]	(2)[b]	(2)[b]	(2)[b]	(2)[b]	
pH	2.7-3.6	3.1, 3.1	(2) 3.1, 3.1	(2) 3.1, 3.1	(2) 3.1, 3.1	(2) 3.1, 3.1	(2) 3.1, 3.1	
Osmolality	Report Results mOsm/kg	(2) 124 124	(2) 124, 124	(2) 127, 127	(2) 128, 128	(2) 128, 128 kg	(2) 124, 124	
Vial Appearance	[d]	(2)[e]	(2)[e]	(2)[e]	(2)[e]	(2)[e]	(2)[e]	
Identification by Retention Time	Retention time between 0.98 and 1.02 relative to standard	1.00 1.00	(2) 1.00 1.00	(2) 1.00 1.00	(2) 1.00 1.00	(2) 1.00 1.00	(2) 1.00 1.00	
Assay	90-110% of label claim	97.8, 97.8	(2) 98.3, 98.3	(2) 99.0, 99.0	(2) 100.1, 100.2	(2) 99.2, 99.2	(2) 97.8, 97.8	

TABLE 12I.2-continued

Long-term stability results (5° C./Ambient) for Formulated (XXXVI), GMP lot# 041I0511[a]

Test	Specification	Months 0	1 (I)*	3 (I)*	6 (I)*	6 (U)**	9 (I)*	10
Related Substances	NMT 1.00% for any single related substance NMT 3.00% total related substances	RRT 0.672: 0.11 RRT 0.672: 0.11 Total: 0.11 Total: 0.11	(2) <0.10%, <0.10% Total: <0.10% Total: <0.10%	(2) <0.10%, <0.10% Total: <0.10% Total: <0.10	(2) <0.10%, <0.10% Total: <0.10% Total: <0.10	(2) <0.10%, <0.10% Total: <0.10% Total: <0.10	(2) RRT 0.672: 0.11 RRT 0.672: 0.11 Total: 0.11 Total: 0.11	
Subvisible Particulates	[f]		(3) 224, 0 150, 0 71, 0	(3) 224, 0 150, 0 71, 0	(3) 27, 0 75, 0 139, 0	(3) 156, 0 150, 0 146, 0		(3) (I)* 133, 0 160, 0 68, 3
Weight Loss (5 vials)	NMT 2.5 g loss in weight	(5) 0.0 all	(5) 0.0 all	(5) 0.0 all	(5) 0.0 all	(5) 0.0 all	(5) 0.0 all	
LAL*	NMT 4 EU/mL							(I) <0.5 EU/mL
Sterility*								

[a]Package components for GMP batch: 50 mL clear, 20 mm opening, USP Type 1 glass (Wheaton 223745); Gray butyl teflon 2 coated stopped. 4416/50 (West 1014 4937); flip off seal, 20 mm, 3766 white, 8-bridge (West 5420 3028).
[b]Colorless to pale yellow liquid. Clear and particle free
[c]Colorless liquid. Clear and particle free
[d]Vial free of cracks, stopper securely in place and no sign of leakage
[e]Both Vials free from crack stoppers securely in place, no sign of leakage
[f]For each container: NMT 6000 ≥ 10 μm particles; NMT 600 ≥ 25 μm
*(I) = inverted;
**(U) = upright

TABLE 12J

Level of Degradant 4,6-Bis(propylamino)-1,3,5-triazin-2-ol (CLXXVIII) GMP StabilityStudy, Lot # GMP lot# 041I0511

	Release Testing (Drug Product Production lot Sample Pull)			T = 1 month		T = 3 month		T = 6 month		T = 9 month	
	Beg.	Mid.	End	Vial 1	Vial 2	Vial 1	Vial 2	Vial 1	Vial 2	Vial 1	Vial 2
5° C., amb. RH Inverted	0.00	0.00	0.00	0.03	0.03	0.06	0.06	0.08	0.08	0.11	0.11
5° C., amb. RH Upright				NA	NA	NA	NA	0.08	0.08	NA	NA

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

What is claimed is:

1. A composition comprising N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXXV) or a salt thereof, wherein 4,6-bis-(n-propylamino)-1,3,5-triazin-2-ol (CLXXVIII) is essentially absent from the composition or is present in the composition at a level equal to or lower than about 1% (w/w) with respect to (XXXV) or the salt thereof.

2. The composition of claim **1**, wherein 4,6-bis-(n-propylamino)-1,3,5-triazin-2-ol (CLXXVIII) is present at a level equal to or lower than about 0.5% (w/w) with respect to (XXXV) or the salt thereof.

3. The composition of claim **2**, wherein 4,6-bis-(n-propylamino)-1,3,5-triazin-2-ol (CLXXVIII) is present at a level equal to or lower than about 0.2% (w/w) with respect to (XXXV) or the salt thereof.

4. The composition of claim **3**, wherein 4,6-bis-(n-propylamino)-1,3,5-triazin-2-ol (CLXXVIII) is present at a level equal to or lower than about 0.15% (w/w) with respect to (XXXV) or the salt thereof.

5. The composition of claim 4, wherein 4,6-bis-(n-propylamino)-1,3,5-triazin-2-ol (CLXXVIII) is present at a level equal to or lower than about 0.1% (w/w) with respect to (XXXV) or the salt thereof.

6. The composition of claim 5, wherein 4,6-bis-(n-propylamino)-1,3,5-triazin-2-ol (CLXXVIII) is essentially absent from the composition.

7. The composition of claim 1, further comprising a buffer and a liquid carrier.

8. The composition of claim 7, wherein the salt of (XXXV) is the hydrogen sulfate salt (XXXVI).

9. The composition of claim 7, further comprising citric acid.

10. The composition of claim 7, wherein the pH of the composition is adjusted with a base.

11. The composition of claim 10, wherein the base comprises sodium hydroxide.

12. The composition of claim 7, wherein the liquid carrier comprises water.

13. The composition of claim 7, wherein the pH of the composition ranges from about 2.5 to about 5.

14. The composition of claim 7, wherein the concentration of (XXXV) or the salt thereof in the composition is about 1-10 mg/mL.

15. The composition of claim 14, wherein the concentration of (XXXV) or the salt thereof in the composition is about 5-10 mg/mL.

16. The composition of claim 15, wherein the concentration of (XXXV) or the salt thereof in the composition is about 10 mg/mL.

17. The composition of claim 7, comprising less than 0.5% (w/w) (CLXXVIII) with respect to (XXXV).

18. The composition of claim 17, wherein less than 0.5% (w/w) of N-(4,6-Bis-n-propylamino)-[1,3,5]-triazin-2-ol (CLXXVIII) with respect to (XXXV) forms over a six-month period of storage of the composition at 2-8° C.

19. The composition of claim 18, wherein less than 0.5% (w/w) of N-(4,6-Bis-n-propylamino)-[1,3,5]-triazin-2-ol (CLXXVIII) with respect to (XXXV) forms over a twenty-four-month period of storage of the composition at 2-8° C.

20. A crystalline free base of N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXXV), Form A, having the XRPD spectrum of FIG. 22.

21. At least one crystalline salt of N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXXV) selected from the group consisting of:

(i) crystalline hydrogen sulfate salt (XXXVI) Form A, having the XRPD spectrum of FIG. 14;

(ii) crystalline hydrogen sulfate salt (XXXVI) comprising a mixture of Form A and Form B, having the XRPD spectrum of FIG. 23;

(iii) crystalline hydrogen sulfate salt (XXXVI) Form C, having the XRPD spectrum of FIG. 24;

(iv) crystalline hydrogen sulfate salt (XXXVI) Form D, having the XRPD spectrum of FIG. 25;

(v) crystalline hydrogen sulfate salt (XXXVI) comprising a mixture of Form A and Form D, having the XRPD spectrum of FIG. 26;

(vi) crystalline phosphoric acid salt (CLXXX) Form A, having the XRPD spectrum of FIG. 27;

(vii) crystalline phosphoric acid salt (CLXXX) Form C, having the XRPD spectrum of FIG. 28;

(viii) crystalline phosphoric acid salt (CLXXX) comprising a mixture of Form A and Form B, having the XRPD spectrum of FIG. 29;

(ix) crystalline phosphoric acid salt (CLXXX) comprising a mixture of Form C and Form D, having the XRPD spectrum of FIG. 30;

(x) crystalline phosphoric acid salt (CLXXX) comprising a mixture of Form C and Form E, having the XRPD spectrum of FIG. 31;

and any mixtures thereof.

22. A salt of N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXXV), comprising about one mole equivalent of sulfuric acid.

23. The salt of claim 22, further comprising one mole equivalent of water.

24. A salt of N-(4,6-Bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXXV), comprising at least one mole equivalent of phosphoric acid.

25. The salt of claim 24, comprising about one mole equivalent of phosphoric acid.

26. A method of increasing minute ventilation in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a composition comprising N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXXV) or a salt thereof, wherein 4,6-bis-(n-propylamino)-1,3,5-triazin-2-ol (CLXXVIII) is essentially absent from the composition or is present in the composition at a level equal to or lower than about 1% (w/w) with respect to (XXXV) or the salt thereof.

27. The method of claim 26, wherein the pharmaceutical formulation is administered via intravenous infusion.

28. The method of claim 26, wherein the infusion dose of the pharmaceutical formulation is at least about 0.016 mg/kg/min of N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXXV).

29. The method of claim 26, wherein the infusion dose of the pharmaceutical formulation is about 0.016 mg/kg/min of N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXXV).

30. The method of claim 26, wherein the infusion dose of the pharmaceutical composition produces a plasma concentrations of at least about 726 ng/mL of N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXXV) in the subject.

31. The method of claim 26, wherein the subject is a mammal.

32. The method of claim 31, wherein the mammal is human.

33. The method of claim 26, wherein the infusion dose of the pharmaceutical formulation is about 0.012 mg/kg/min of N-(4,6-bis-n-propylamino-[1,3,5]triazin-2-yl)-N,O-dimethyl-hydroxylamine (XXXV).

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